

# Novocastra™ Liquid Mouse Monoclonal Antibody Estrogen Receptor

**Product Code: NCL-L-ER-6F11**

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EN FR IT DE ES PT  
SV EL DA NL NO TR

## Instructions for Use

Please read before using this product.

## Mode d'emploi

À lire avant d'utiliser ce produit.

## Istruzioni per l'uso

Si prega di leggere, prima di usare il prodotto.

## Gebrauchsanweisung

Bitte vor der Verwendung dieses Produkts lesen.

## Instrucciones de uso

Por favor, leer antes de utilizar este producto.

## Instruções de Utilização

Leia estas instruções antes de utilizar este produto.

## Bruksanvisning

Var god läs innan ni använder produkten.

## Οδηγίες Χρήσης

Παρακαλούμε διαβάστε τις οδηγίες πριν χρησιμοποιήσετε το προϊόν αυτό.

## Brugsanvisning

Læs venligst før produktet tages i brug.

## Gebruiksaanwijzing

Lezen vóór gebruik van dit product.

## Bruksanvisning

Vennligst les denne før du bruker produktet.

## Kullanım Talimatları

Lütfen bu ürünü kullanmadan önce okuyunuz.

## Check the integrity of the packaging before use.

Vérifier que le conditionnement est en bon état avant l'emploi.

Prima dell'uso, controllare l'integrità della confezione.

Vor dem Gebrauch die Verpackung auf Unversehrtheit überprüfen.

Comprobar la integridad del envase, antes de usarlo.

Verifique a integridade da embalagem antes de utilizar o produto.

Kontrollera att paketet är obrutet innan användning.

Ελέγξτε την ακεραιότητα της συσκευασίας πριν από τη χρήση.

Kontroller, at pakken er ubeskadiget før brug.

Controleer de verpakking vóór gebruik.

Sjekk at pakningen er intakt før bruk.

Kullanmadan önce ambalajın bozulmamış olmasını kontrol edin.

**www.LeicaBiosystems.com**



# Novocastra™ Liquid Mouse Monoclonal Antibody Estrogen Receptor

## Product Code: NCL-L-ER-6F11

### Intended Use

*For in vitro diagnostic use.*

NCL-L-ER-6F11 is intended for the qualitative identification by light microscopy of Estrogen Receptor molecules in paraffin sections. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

### Principle of Procedure

Immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

### Clone

6F11

### Immunogen

Prokaryotic recombinant protein corresponding to the full length alpha form of the human estrogen receptor molecule.

### Specificity

Human estrogen receptor.

### Reagent Composition

NCL-L-ER-6F11 is a liquid tissue culture supernatant containing sodium azide as a preservative.

### Ig Class

IgG1

### Total Protein Concentration

Total Protein

Refer to vial label for lot specific total protein concentration.

### Antibody Concentration

Greater than or equal to 67.5 mg/L as determined by ELISA. Refer to vial label for lot specific Ig concentration.

### Recommendations On Use

Immunohistochemistry on paraffin sections.

**Heat Induced Epitope Retrieval (HIER):** Please follow the instructions for use in Novocastra Epitope Retrieval Solution pH 6.

**Suggested dilution:** 1:50 for 30 minutes at 25 °C. This is provided as a guide and users should determine their own optimal working dilutions.

**Visualization:** Please follow the instructions for use in the Novolink™ Polymer Detection Systems. For further product information or support, contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems' Web site, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

The performance of this antibody should be validated when utilized with other manual staining systems or automated platforms.

### Storage and Stability

Store at 2–8 °C. Do not freeze. Return to 2–8 °C immediately after use. Do not use after expiration date indicated on the vial label. Storage conditions other than those specified above must be verified by the user.

### Specimen Preparation

The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

### Warnings and Precautions

This reagent has been prepared from the supernatant of cell culture. As it is a biological product, reasonable care should be taken when handling it.

This reagent contains sodium azide. A Material Safety Data Sheet is available upon request or available from [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Consult federal, state or local regulations for disposal of any potentially toxic components.

Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.† Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.

Minimize microbial contamination of reagents or an increase in non-specific staining may occur.

Incubation times or temperatures, other than those specified, may give erroneous results. Any such changes must be validated by the user.

## Quality Control

Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures.

Controls should be fresh autopsy/biopsy/surgical specimens, formalin-fixed, processed and paraffin wax-embedded as soon as possible in the same manner as the patient sample(s).

## Positive Tissue Control

Used to indicate correctly prepared tissues and proper staining techniques.

One positive tissue control should be included for each set of test conditions in each staining run.

A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation.<sup>2</sup>

Recommended positive control tissue is endometrium.

If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

## Negative Tissue Control

Should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody.

Recommended negative control tissue is endothelial elements of tonsil.

Alternatively, the variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user.

Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain non-specifically.<sup>3</sup> False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by endogenous enzymes such as pseudoperoxidase (erythrocytes), endogenous peroxidase (cytochrome C), or endogenous biotin (eg. liver, breast, brain, kidney) depending on the type of immunostain used. To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate chromogen or enzyme complexes (avidin-biotin, streptavidin, labeled polymer) and substrate-chromogen, respectively. If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid.

## Negative Reagent Control

Use a non-specific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site.

## Patient Tissue

Examine patient specimens stained with NCL-L-ER-6F11 last. Positive staining intensity should be assessed within the context of any non-specific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

## Results Expected

### Normal Tissues

Clone 6F11 detects the estrogen receptor (ER) alpha antigen in the nuclei of cells that express high levels of ER, including a proportion of endometrial, ovarian and myometrial cells, and normal breast ductal cells. Staining may also be seen in tonsil mucosa and a proportion of germinal center lymphoid cells. (Total number of normal cases evaluated = 136).

### Abnormal Tissues

Clone 6F11 stained 137/222 tumors evaluated, including breast tumors (131/180), thyroid papillary carcinomas (3/4), ovarian tumors (2/4, including 1/2 cystadenocarcinomas and 1/1 clear cell carcinomas), metastatic tumors of unknown origin (1/2), lung tumors (0/4), liver tumors (0/4), brain tumors (0/2), squamous cell carcinomas of the esophagus (0/2), stomach adenocarcinomas (0/2), soft tissue tumors (0/2), squamous cell carcinomas of the tongue (0/2), renal cell carcinomas (0/2), squamous cell carcinomas of the cervix (0/2), testicular seminomas (0/2), colon adenocarcinomas (0/2), rectal adenocarcinomas (0/2), skin tumors (0/2), squamous cell carcinomas of the larynx (0/1) and atypical carcinoid tumors of the thymus (0/1). (Total number of tumor cases evaluated = 222).

**NCL-L-ER-6F11 is recommended for determining the estrogen receptor alpha status of breast cancer tissues.**

## General Limitations

Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.

Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.<sup>4</sup> Excessive or incomplete counterstaining may compromise proper interpretation of results.

The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Antibodies from Leica Biosystems Newcastle Ltd are for use, as indicated, on either frozen or paraffin-embedded sections with specific fixation requirements. Unexpected antigen expression may occur, especially in neoplasms. The clinical interpretation of any stained tissue section must include morphological analysis and the evaluation of appropriate controls.

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## Amendments to Previous Issue

Recommendations On Use, Results Expected.

## Date of Issue

20 September 2012

# Novocastra™ Anticorps Monoclonal Liquide de Souris

## Estrogen Receptor

### Référence du Produit: NCL-L-ER-6F11

#### Utilisation Prévue

*Diagnostic in vitro.*

Le NCL-L-ER-6F11 est destiné à l'identification qualitative par microscopie optique de la molécule Estrogen Receptor sur des coupes en paraffine. L'interprétation clinique de tout marquage, ou absence de marquage, doit être complétée par des études morphologiques utilisant des contrôles appropriés et doit être évaluée par un pathologiste qualifié à la lumière des antécédents cliniques du patient et d'autres analyses diagnostiques.

#### Principe de la Procédure

Les techniques de marquage immunohistochimique (IHC) permettent la visualisation des antigènes via l'application séquentielle d'un anticorps spécifique sur un antigène (anticorps primaire), d'un anticorps secondaire sur l'anticorps primaire et d'un complexe enzymatique comportant un substrat chromogène, avec des étapes de lavage intercalées. L'activation enzymatique du chromogène se traduit par la présence d'un produit de réaction visible au niveau du site de l'antigène. Le spécimen peut ensuite faire l'objet d'une coloration de contraste et être placé sous une lamelle. Les résultats sont interprétés à l'aide d'un microscope optique et participent au diagnostic différentiel des processus physiopathologiques, susceptibles, ou non, d'être associés à un antigène particulier.

#### Clone

6F11

#### Immunogène

Protéine procaryote recombinante correspondant à la longueur totale de la forme alpha de la molécule du récepteur des œstrogènes humains.

#### Spécificité

Récepteur des œstrogènes humains.

#### Composition du Réactif

Le NCL-L-ER-6F11 est un surageant de culture tissulaire liquide contenant une solution d'azide de sodium comme conservateur.

#### Classe d'Ig

IgG1

#### Concentration Totale en Protéines

Total Protein

La concentration totale en protéines, spécifique du lot, figure sur l'étiquette du flacon.

#### Concentration en Anticorps

Supérieure ou égale à 67,5 mg/l, déterminée par la méthode ELISA. La concentration totale en Ig, spécifique du lot, figure sur l'étiquette du flacon.

#### Recommandations d'utilisation

Immunohistochimie sur coupes en paraffine.

**Restauration d'épitope induite par la chaleur (HIER):** Veuillez respecter le mode d'emploi de Novocastra Epitope Retrieval Solution pH 6.

**Dilution préconisée:** 1:50 durant 30 minutes à 25 °C. Ceci n'est donné qu'à titre indicatif et les utilisateurs doivent déterminer leurs propres dilutions de travail optimales.

**Visualisation:** Veuillez respecter le mode d'emploi des Novolink™ Polymer Detection Systems. Pour plus d'informations sur le produit ou pour toute assistance, contactez votre représentant local ou le bureau régional de Leica Biosystems, ou sinon rendez vous sur le site [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com) de Leica Biosystems.

Les performances de cet anticorps devront être validées lorsqu'il est utilisé avec d'autres systèmes de coloration manuels ou plates-formes automatisées.

#### Conservation et Stabilité

Conserver à 2–8 °C. Ne pas congeler. Remettre immédiatement à 2–8 °C après utilisation. Ne pas utiliser après la date de péremption indiquée sur l'étiquette du récipient. Les conditions de conservation autres que celles qui sont spécifiées ci-dessus doivent faire l'objet d'une vérification par l'utilisateur.

#### Préparation des Spécimens

Le fixateur recommandé est le formol à 10%, tamponné, neutre, pour coupes tissulaires incluses en paraffine.

#### Mises en Garde et Précautions

Ce réactif a été préparé à partir du surageant d'une culture cellulaire. Du fait de sa nature de produit biologique, sa manipulation doit faire l'objet du plus grand soin.

Ce réactif contient de l'azide de sodium. Une Fiche de données de sécurité est disponible sur demande ou sur le site [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Consulter les réglementations nationales, régionales ou locales en vigueur relatives à l'élimination de tous les éléments potentiellement toxiques.

Les spécimens, avant et après fixation, ainsi que toutes les matières ayant été en contact avec eux, doivent être manipulés comme s'ils étaient susceptibles de transmettre une infection et être éliminés en respectant les précautions appropriées<sup>1</sup>. Ne jamais pipeter les réactifs avec la bouche et éviter tout contact des réactifs et des spécimens avec la peau et les membranes muqueuses. Rincer avec de grandes quantités d'eau en cas de contact des réactifs ou des spécimens avec des zones sensibles. Consulter un médecin.

Minimiser la contamination microbienne des réactifs sinon un accroissement du marquage non spécifique est susceptible de se produire.

Des durées et des températures d'incubation différentes de celles qui ont été spécifiées sont susceptibles de conduire à des résultats erronés. Toutes les modifications doivent être validées par l'utilisateur.

### **Contrôle de Qualité**

Des différences de traitement des tissus et de procédures techniques du laboratoire de l'utilisateur sont susceptibles de conduire à une variabilité significative des résultats, ce qui rend nécessaire la mise en œuvre de contrôles en interne en plus des procédures suivantes.

Les contrôles doivent être des spécimens frais provenant d'autopsies, de biopsies ou d'interventions chirurgicales, fixés au formol, traités et inclus en cire de paraffine dès que possible, de la même façon que le(s) échantillon(s) de patient.

### **Tissu de Contrôle Positif**

Il est utilisé pour indiquer que les tissus ont été préparés correctement et que les techniques de marquage étaient appropriées.

Un contrôle tissulaire positif doit être inclus dans toute opération de marquage pour chaque ensemble de conditions d'analyse.

Un tissu présentant un marquage faiblement positif est plus adapté à un contrôle de qualité optimal qu'un tissu présentant un marquage fortement positif et il permet de détecter de moindres niveaux de dégradation du réactif.<sup>2</sup>

Le tissu de contrôle positif recommandé est L'endomètre.

Si le tissu de contrôle positif ne présente pas de marquage positif, les résultats des spécimens analysés doivent être considérés comme invalides.

### **Tissu de Contrôle Négatif**

Il doit être examiné après le tissu de contrôle positif afin de vérifier la spécificité du marquage de l'antigène cible par l'anticorps primaire.

Les amygdales constitue le tissu de contrôle négatif recommandé.

Si non, la diversité des types cellulaires présents dans la plupart des tissus permet de disposer fréquemment de sites de contrôle négatif, mais ils doivent être vérifiés par l'utilisateur.

S'il est présent, le marquage non spécifique prend habituellement une apparence diffuse. Un marquage sporadique du tissu conjonctif peut également être observé sur des coupes de tissus qui ont été fixées par un excès de formol. Utiliser des cellules intactes pour l'interprétation des résultats du marquage. Les cellules nécrotiques ou dégénérées sont souvent marquées de façon non spécifique.<sup>3</sup>

Des résultats faussement positifs peuvent être observés en raison d'une liaison non immunologique à des protéines ou à des produits de réaction du substrat. Ils peuvent également être provoqués par des enzymes endogènes comme la pseudoperoxydase (érythrocytes), la peroxydase endogène (cytochrome C), ou la biotine endogène (foie, sein, cerveau, rein, par exemple) selon le type d'immunomarquage utilisé. Pour différencier l'activité des enzymes endogènes ou la liaison non spécifique d'enzymes de l'immunoréactivité spécifique, des tissus supplémentaires du patient peuvent être marqués exclusivement avec le substrat chromogène ou par des complexes enzymatiques (avidine-biotine, streptavidine, polymère marqué) et le substrat chromogène respectivement. Si un marquage spécifique se produit dans le tissu de contrôle négatif, les résultats des spécimens du patient doivent être considérés comme invalides.

### **Réactif de Contrôle Négatif**

Utiliser un réactif de contrôle négatif non spécifique à la place de l'anticorps primaire avec une coupe de chaque spécimen du patient afin d'évaluer le marquage non spécifique et de permettre une meilleure interprétation du marquage spécifique au niveau du site antigénique.

### **Tissu du Patient**

Examiner les échantillons du patient marqués au NCL-L-ER-6F11 en dernier lieu. L'intensité du marquage positif doit être évaluée à la lumière du bruit de fond du marquage non spécifique du réactif de contrôle négatif. Comme pour toutes les analyses immunohistochimiques, un résultat négatif signifie que l'antigène n'a pas été détecté mais ne signifie pas qu'il est absent des cellules/tissus testés. Si nécessaire, employer un panel d'anticorps pour identifier les réactions faussement négatives.

### **Résultats Attendus**

#### Tissus normaux

Le clone 6F11 détecte l'antigène alpha du récepteur des œstrogènes (RO) dans le noyau des cellules qui expriment des niveaux élevés de RO, y compris une proportion des cellules endométriales, ovariennes et myométriques et des cellules canalaire mammaires normales. Le marquage peut aussi être constaté dans la muqueuse des amygdales et une proportion de cellules lymphoïdes du centre germinatif. (Nombre total de cas normaux évalués = 136).

#### Tissus tumoraux

Le clone 6F11 a marqué 137/222 tumeurs évaluées, dont des tumeurs du sein (131/180), des carcinomes papillaires de la thyroïde (3/4), des tumeurs de l'ovaire (2/4, dont 1/2 cystadénocarcinomes et 1/1 carcinome à cellules claires), des tumeurs métastatiques d'origine inconnue (1/2), des tumeurs du poulmon (0/4), des tumeurs du foie (0/4), des tumeurs cérébrales (0/2), des carcinomes squameux de l'œsophage (0/2), des adénocarcinomes gastriques (0/2), des tumeurs des tissus mous (0/2), des carcinomes squameux de la langue (0/2), des carcinomes à cellules rénales (0/2), des carcinomes squameux du col utérin (0/2), des séminomes testiculaires (0/2), des adénocarcinomes du côlon (0/2), des adénocarcinomes du rectum (0/2), des tumeurs de la peau (0/2), des carcinomes squameux du larynx (0/1) et des tumeurs carcinoides atypiques du thymus (0/1). (Nombre total de cas de tumeur évalués = 222).

**L'utilisation du NCL-L-ER-6F11 est recommandée dans le cadre de la détermination du statut des tissus mammaires cancéreux vis-à-vis du récepteur alpha des œstrogènes.**

### **Limites Générales**

L'immunohistochimie est un processus diagnostique constitué de plusieurs étapes qui nécessite une formation spécialisée relative au choix des réactifs appropriés ; au choix, à la fixation et au traitement des tissus ; à la préparation des lames IHC ; et à l'interprétation des résultats du marquage.

Le marquage des tissus dépend de leur manipulation et de leur traitement avant le marquage. Une fixation, une congélation, une décongélation, un lavage, un séchage, un chauffage, une coupe, incorrects ou une contamination par d'autres tissus ou d'autres liquides sont susceptibles de conduire à la production d'artefacts, au piégeage de l'anticorps ou à des résultats faussement négatifs. Des variations dans les méthodes de fixation et d'inclusion, ainsi que des irrégularités propres au tissu, peuvent conduire à des résultats incohérents.<sup>4</sup>

Une coloration de contraste excessive ou incomplète peut gêner l'interprétation correcte des résultats.

L'interprétation clinique de tout marquage, ou absence de marquage, doit être complétée par des études morphologiques utilisant des contrôles appropriés et doit être évaluée par un pathologiste qualifié à la lumière des antécédents cliniques du patient et d'autres analyses diagnostiques.

Les anticorps de Leica Biosystems Newcastle Ltd sont destinés, selon les besoins, à être utilisés sur des coupes incluses en paraffine ou des coupes congelées, et conformément à des exigences particulières en matière de fixation. Une expression antigénique inattendue est susceptible de se produire, en particulier au niveau des néoplasmes. L'interprétation clinique de toute coupe tissulaire marquée doit comporter une analyse morphologique et l'évaluation des contrôles appropriés.

## Bibliographie Générale

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## Amendements Apportés à la Version Précédente

Recommandations d'utilisation, Résultats Attendus.

## Date de Publication

20 septembre 2012



# Novocastra™ Anticorpo Monoclonale Murino Liquido

## Estrogen Receptor

### Codice Del Prodotto: NCL-L-ER-6F11

#### Uso Previsto

Per uso diagnostico in vitro.

NCL-L-ER-6F11 è destinato all'identificazione qualitativa in microscopia ottica della molecola Estrogen Receptor, in sezioni incluse in paraffina. L'interpretazione clinica di ogni colorazione o della sua assenza va integrata da studi morfologici che utilizzino i controlli appropriati e deve essere valutata da un patologo qualificato, nel contesto della storia clinica del paziente e delle altre metodiche diagnostiche adoperate.

#### Principio Della Procedura

Le tecniche di colorazione immunostochimica (IHC) consentono la visualizzazione degli antigeni mediante l'applicazione sequenziale di un anticorpo specifico per l'antigene (anticorpo primario), di un anticorpo secondario che lega l'anticorpo primario e di un complesso enzimatico con un substrato cromogeno; l'applicazione dei tre reagenti è intervallata da fasi di lavaggio. L'attivazione enzimatica del cromogeno produce una reazione visibile in corrispondenza del sito antigenico. Il campione biologico può, quindi, essere controcolorato e montato. I risultati vengono interpretati mediante un microscopio ottico e sono utili nella diagnosi differenziale di processi fisiopatologici, che possono essere più o meno associati ad un particolare antigene.

#### Clone

6F11

#### Immunogeno

Proteina ricombinante procariotica, corrispondente alla forma alfa completa (full-length) della molecola recettoriale estrogenica umana.

#### Specificità

Recettore estrogenico umano.

#### Composizione Del Reagente

NCL-L-ER-6F11 è un supernatante liquido di coltura tissutale, contenente di sodio azide come conservante.

#### Classe Ig

IgG1

#### Concentrazione Proteica Totale

Total Protein

Consultare l'etichetta del flacone per la concentrazione proteica totale specifica del lotto.

#### Concentrazione Anticorpale

Superiore o uguale a 67,5 mg/l, come determinato mediante test ELISA. Consultare l'etichetta del flacone per la concentrazione di Ig specifica del lotto.

#### Raccomandazioni Per L'uso

Immunostochimica su sezioni incluse in paraffina.

**Smascheramento antigenico termoidotto (HIER):** Si prega di seguire le istruzioni per l'uso fornite in Novocastra Epitope Retrieval Solution pH 6.

**Diluizione raccomandata:** 1:50 per 30 minuti a 25 °C. Queste raccomandazioni costituiscono delle semplici linee guida; spetta al singolo utente stabilire le diluizioni di lavoro ottimali.

**Visualizzazione:** Si raccomanda di seguire le istruzioni per l'uso dei Novolink™ Polymer Detection Systems. Per ulteriori informazioni sui prodotti o assistenza, contattare il distributore di zona o la sede regionale di Leica Biosystems, oppure visitare il sito internet di Leica Biosystems, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

La resa di questo anticorpo deve essere validata quando viene utilizzato con altri metodi di colorazione manuale o piattaforme automatizzate.

#### Conservazione E Stabilità

Conservare a 2–8 °C. Non congelare. Immediatamente dopo l'uso, raffreddare di nuovo a 2–8 °C. Non usare dopo la data di scadenza, indicata sull'etichetta del flacone. Condizioni di conservazione diverse da quelle sopra specificate vanno verificate dall'utente.

#### Preparazione Del Campione Biologico

Il fissativo raccomandato è la formalina tamponata neutra al 10% per sezioni tissutali incluse in paraffina.

#### Avvertenze E Precauzioni

Questo reagente è stato preparato dal supernatante di coltura cellulare. Trattandosi di un prodotto biologico, va maneggiato con cautela. Questo reagente contiene sodio azide. Una scheda di sicurezza del prodotto (MSDS) è disponibile su richiesta o dal sito [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Fare riferimento alla normativa federale, statale o locale per lo smaltimento dei componenti potenzialmente tossici.

Prima e dopo la fissazione, i campioni biologici e tutti i materiali ad essi esposti vanno trattati come potenzialmente infettanti e smaltiti con le appropriate precauzioni. Non pipettare i reagenti con la bocca ed evitare il contatto dei reagenti e dei campioni biologici con la pelle e con le mucose. Se i reagenti o i campioni biologici vengono a contatto con zone sensibili, sciacquare abbondantemente le parti interessate. Consultare il medico.

Ridurre al minimo la contaminazione microbica dei reagenti, allo scopo di evitare un aumento di colorazione aspecifica.

Tempi o temperature d'incubazione diversi da quelli specificati possono condurre a risultati non veritieri. Tali variazioni devono essere convalidate dall'utente.

### **Controllo Qualità**

Differenze nella processazione del tessuto e nelle tecniche in uso presso il laboratorio dell'utente possono produrre una discrepanza significativa nei risultati, rendendo necessaria la regolare esecuzione di controlli interni, in aggiunta alle procedure descritte di seguito.

I controlli devono essere costituiti da campioni biologici freschi autoptici/bioptici/chirurgici e devono essere il più rapidamente possibile fissati in formalina, processati ed inclusi in paraffina, allo stesso modo dei campioni biologici ottenuti dal paziente.

### **Controllo Positivo Del Tessuto**

È usato per indicare tessuti correttamente preparati e tecniche di colorazione appropriate.

Per ogni gruppo di condizioni del test e ogni volta che viene eseguita la colorazione, deve essere incluso un controllo positivo del tessuto.

Un tessuto a debole colorazione positiva è più adatto di uno a colorazione positiva intensa per un ottimale controllo qualità e per mettere in evidenza anche minimi livelli di degradazione del reagente.<sup>2</sup>

Il tessuto raccomandato per il controllo positivo è l'endometrio.

Se il controllo positivo del tessuto non dimostra colorazione positiva, i risultati con i campioni biologici del test vanno considerati non validi.

### **Controllo Negativo Del Tessuto**

Va esaminato dopo il controllo positivo, per verificare la specificità nei confronti dell'antigene bersaglio da parte dell'anticorpo primario.

Il tessuto raccomandato per il controllo negativo è la tonsilla.

In alternativa, la varietà dei tipi cellulari presenti nella maggior parte delle sezioni tissutali offre spesso siti di controllo negativo, ma questo va verificato dall'utente.

La colorazione aspecifica, se presente, assume di solito aspetto diffuso. La colorazione sporadica del tessuto connettivo può anche manifestarsi in seguito ad iperfissazione di sezioni di tessuto in formalina. Per l'interpretazione dei risultati della colorazione, usare cellule intatte. Le cellule necrotiche o degenerate si colorano spesso in maniera aspecifica<sup>3</sup>. Si possono osservare risultati falsamente positivi, dovuti a legame non immunologico delle proteine o a prodotti di reazione del substrato. Tali falsi positivi possono essere anche causati da enzimi endogeni quali la pseudoperossidasi (eritrociti), la perossidasi endogena (citocromo C) o la biotina endogena (es. fegato, mammella, cervello, rene), a seconda del tipo di immunocolorazione usato. Per differenziare l'attività enzimatica endogena o il legame enzimatico aspecifico dall'immunoreattività specifica, possono essere colorati ulteriori tessuti del paziente esclusivamente con substrato cromogeno o con complessi enzimatici (avidina-biotina, streptavidina, polimero marcato) e substrato cromogeno. Se nel controllo negativo del tessuto compare una colorazione specifica, i risultati sui campioni biologici ottenuti dal paziente devono essere considerati non validi.

### **Controllo Negativo Del Reagente**

Usare un controllo negativo aspecifico del reagente in luogo dell'anticorpo primario, con una sezione di ogni campione biologico del paziente, per valutare la colorazione aspecifica e per consentire una migliore interpretazione della colorazione specifica in corrispondenza del sito antigenico.

### **Tessuto Del Paziente**

Successivamente, esaminare i campioni biologici del paziente colorati con NCL-L-ER-6F11. L'intensità della colorazione positiva va analizzata nel contesto di qualsiasi colorazione aspecifica di fondo del controllo negativo del reagente. Come per tutti gli altri test immunistochimici, un risultato negativo significa che l'antigene non è stato determinato, ma non necessariamente che fosse assente dalle cellule o dal tessuto esaminato. Se necessario, usare un pannello di anticorpi per identificare reazioni falsamente negative.

### **Risultati Attesi**

#### Tessuti normali

Il clone 6F11 rileva l'antigene alfa del recettore dell'estrogeno (ER) nei nuclei delle cellule che esprimono livelli elevati di ER, tra cui una proporzione di cellule endometriali, ovariche e miometriali e cellule duttali della mammella normale. È possibile osservare inoltre la colorazione nella mucosa tonsillare e in una proporzione di cellule linfoidi del centro germinale. (Numero complessivo di casi normali valutati = 136).

#### Tessuti tumorali

Il clone 6F11 ha colorato 137/222 tumori valutati, tra cui tumori della mammella (131/180), carcinomi papillari della tiroide (3/4), tumori dell'ovario (2/4, inclusi 1/2 cistoadenocarcinomi e 1/1 carcinomi a cellule chiare), tumori metastatici di origine sconosciuta (1/2), tumori del polmone (0/4), tumori del fegato (0/4), tumori del cervello (0/2), carcinomi a cellule squamose dell'esofago (0/2), adenocarcinomi dello stomaco (0/2), tumori dei tessuti molli (0/2), carcinomi a cellule squamose della lingua (0/2), carcinomi a cellule renali (0/2), carcinomi a cellule squamose della cervice (0/2), seminomi testicolari (0/2), adenocarcinomi del colon (0/2), adenocarcinomi rettali (0/2), tumori della pelle (0/2), carcinomi a cellule squamose della laringe (0/1) e tumori carcinoidi atipici del timo (0/1). (Numero complessivo di casi di tumori valutati = 222).

**Si raccomanda l'uso di NCL-L-ER-6F11 nella determinazione dello stato del recettore estrogenico alfa nel tessuto canceroso mammario.**

### **Limitazioni Generali**

L'immunistochimica è un procedimento diagnostico a più passi (multistep) che richiede un'esperienza specifica nella selezione dei reagenti appropriati, nella selezione, fissazione e processazione dei tessuti, nella preparazione di vetrini IHC e nell'interpretazione dei risultati della colorazione.

La colorazione del tessuto dipende dalle modalità di manipolazione e di processazione del tessuto stesso, adottate prima della colorazione. La fissazione, il congelamento, lo scongelamento, il lavaggio, l'asciugatura, il riscaldamento o la sezione condotti in modo non corretto, o la contaminazione con altri tessuti o liquidi, possono produrre artefatti, intrappolamento (trapping) anticorpale o risultati falsi negativi. Risultati incompatibili possono essere dovuti a modifiche dei metodi di fissazione e di inclusione o ad irregolarità intrinseche al tessuto.<sup>4</sup>

Una controcolorazione eccessiva o incompleta può compromettere la corretta interpretazione dei risultati.

L'interpretazione clinica di ogni colorazione o della sua assenza va integrata da studi morfologici che utilizzino i controlli appropriati e deve essere valutata da un patologo qualificato, nel contesto della storia clinica del paziente e delle altre metodiche diagnostiche adoperate.

Gli anticorpi di Leica Biosystems Newcastle Ltd. sono destinati all'uso, quando indicato, su sezioni congelate o incluse in paraffina, con specifici requisiti di fissazione. Un'espressione antigenica inattesa può manifestarsi in particolare nelle neoplasie. L'interpretazione clinica di ogni sezione tissutale colorata deve includere l'analisi morfologica e la valutazione dei controlli appropriati.

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## Modifiche Alla Pubblicazione Precedente

Raccomandazioni Per L'uso, Risultati Attesi.

## Data Di Pubblicazione

20 settembre 2012

# Novocastra™ Flüssiger Monoklonaler Maus-Antikörper

## Estrogen Receptor

### Produkt-Nr.: NCL-L-ER-6F11

#### Verwendungszweck

Für *in-vitro*-Diagnostik.

NCL-L-ER-6F11 ist für den qualitativen Nachweis der Estrogen Receptor-Moleküle in Paraffinschnitten mittels Lichtmikroskopie gedacht. Die klinische Bewertung einer vorliegenden bzw. fehlenden Färbung sollte durch morphologische Studien mit entsprechenden Kontrollen ergänzt und im Kontext der Krankengeschichte des Patienten und anderer diagnostischer Tests von einem qualifizierten Pathologen vorgenommen werden.

#### Verfahrensgrundlage

Immunhistochemische (IHC) Färbetechniken gestatten die optische Darstellung von Antigenen mittels sequentieller Anwendung eines spezifischen Antikörpers zum Antigen (primärer Antikörper), eines sekundären Antikörpers zum primären Antikörper und eines Enzymkomplexes mit einem chromogenen Substrat, jeweils getrennt durch dazwischen liegende Waschschriffe. Die enzymatische Aktivierung des Chromogens führt zu einem sichtbaren Reaktionsprodukt am Ort des Antigens. Die Probe kann dann gegengefärbt und mit einem Deckglas versehen werden. Die Ergebnisse werden mithilfe eines Lichtmikroskops interpretiert und unterstützen die Differentialdiagnose pathophysiologischer Prozesse, die mit einem bestimmten Antigen assoziiert sein könnten.

#### Klon

6F11

#### Immunogen

Prokaryotisches rekombinantes Protein, das der Alpha-Form (in voller Länge) des humanen Östrogenrezeptor-Moleküls entspricht.

#### Spezifität

Humaner Östrogenrezeptor.

#### Reagenzzusammensetzung

NCL-L-ER-6F11 ist ein flüssiger Gewebekulturüberstand, derol/ Natriumazid als Konservierungsmittel enthält.

#### Ig-Klasse

IgG1

#### Gesamtproteinkonzentration Total Protein

Siehe Angaben auf dem Produktetikett bezüglich der chargenspezifischen Gesamtproteinkonzentration.

#### Antikörperkonzentration

Größer als oder gleich 67,5 mg/l laut ELISA-Bestimmung. Hinsichtlich der chargenspezifischen Ig-Konzentration siehe Angaben auf dem Produktetikett.

#### Gebrauchsempfehlungen

Immunhistochemie in Paraffinschnitten

**Hitzeinduzierte Epitopdemaskierung (HIER):** Bitte Gebrauchsanweisung für Novocastra Epitope Retrieval Solution pH 6 befolgen.

**Empfohlene Verdünnung:** 1:50 über einen Zeitraum von 30 Minuten bei 25 °C. Dies ist nur eine Empfehlung, und die Benutzer sollten ihre eigenen optimalen Arbeitsverdünnungen bestimmen.

**Visualisierung:** Bitte Gebrauchsanweisung für Novolink™ Polymer Detection Systems befolgen. Wenn Sie weitere Produktinformationen oder Unterstützung wünschen, setzen Sie sich bitte mit ihrem Händler vor Ort oder mit der Zweigniederlassung von Leica Biosystems in Verbindung beziehungsweise besuchen Sie die Internetseite von Leica Biosystems, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Die Leistungsfähigkeit dieses Antikörpers sollte bestätigt werden, wenn er mit anderen manuellen Färbesystemen oder automatisierten Plattformen eingesetzt wird.

#### Lagerung und Stabilität

Bei 2–8 °C lagern. Nicht einfrieren. Nach Gebrauch sofort wieder bei 2–8 °C lagern. Nach Ablauf des Verfallsdatums (auf dem Behälteretikett) darf das Produkt nicht mehr verwendet werden. Lagerbedingungen, die von den oben genannten Bedingungen abweichen, müssen vom Benutzer verifiziert werden.

#### Probenvorbereitung

Für paraffineingebettete Gewebeschnitte ist das empfohlene Fixativ 10% neutral gepuffertes Formalin.

#### Warnhinweise und Sicherheitsmaßnahmen

Dieses Reagenz wurde aus Zellkulturüberstand zubereitet. Das Reagenz ist ein biologisches Produkt und sollte mit entsprechender Vorsicht gehandhabt werden.

Dieses Reagenz enthält Natriumazid. Ein Material Sicherheits-Datenblatt ist auf Anfrage von [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com) erhältlich. Die entsprechenden nationalen und lokalen Bestimmungen und Vorschriften zur Entsorgung potentiell giftiger Komponenten sind einzuhalten.

Vor und nach der Fixierung sind die Proben sowie alle Materialien, die mit ihnen in Kontakt gekommen sind, als potentiell infektiös zu behandeln und daher mit entsprechender Vorsicht zu entsorgen.<sup>1</sup> Reagenzien dürfen niemals mit dem Mund pipettiert werden, und jeglicher Kontakt der Reagenzien und Proben mit Haut und Schleimhäuten ist zu vermeiden. Falls Reagenzien oder Proben mit empfindlichen Bereichen in Kontakt gekommen sind, müssen diese mit reichlich Wasser gespült werden. Ärztlichen Rat einholen. Die mikrobielle Verunreinigung von Reagenzien ist zu minimieren, da ansonsten eine erhöhte unspezifische Färbung auftreten kann. Falls die spezifizierten Inkubationszeiten oder –temperaturen nicht eingehalten werden, kann es zu fehlerhaften Ergebnissen kommen. Jegliche Abweichungen von den angegebenen Werten müssen vom Benutzer verifiziert werden.

### **Qualitätskontrolle**

Unterschiede bei der Gewebeparbeitung und den technischen Verfahren im Labor des Benutzers können zu signifikanten Schwankungen bei den Ergebnissen führen. Daher ist es wichtig, zusätzlich zu den folgenden Verfahren regelmäßige laborinterne Kontrollen durchzuführen.

Die Kontrollen sollten mit frischen Autopsie-/Biopsie-/chirurgischen Proben vorgenommen werden, die so bald wie möglich und auf dieselbe Weise wie die Patientenprobe(n) in Formalin fixiert, behandelt und in Paraffin eingebettet worden sind.

### **Positive Gewebekontrolle**

Zeigt korrekt vorbereitete Gewebe und korrekte Färbetechniken an.

In jedem Färbelauf sollte für jeden Satz Testbedingungen eine positive Gewebekontrolle durchgeführt werden.

Gewebe mit schwach positiver Färbung ist für die optimale Qualitätskontrolle und den Nachweis kleiner Minderungen in der Reagenzleistung besser geeignet als ein Gewebe mit stark positiver Färbung.<sup>2</sup>

Für die positive Gewebekontrolle wird Endometrium-Gewebe empfohlen.

Falls das positive Kontrollgewebe keine positive Färbung nachweisen kann, sollten die mit den Testproben erzielten Ergebnisse als ungültig betrachtet werden.

### **Negative Gewebekontrolle**

Die negative Gewebekontrolle sollte nach der positiven Gewebekontrolle erfolgen, um die Spezifität der Zielantigenmarkierung durch den primären Antikörper zu verifizieren.

Für die negative Gewebekontrolle wird Tonsillengewebe empfohlen.

Alternativ bietet die Vielfalt unterschiedlicher Zelltypen, die in den meisten Gewebeschnitten vorliegen, häufig Stellen für eine negative Kontrolle. Jedoch sollte dies vom Benutzer verifiziert werden.

Liegt eine unspezifische Färbung vor, hat diese gewöhnlich ein diffuses Erscheinungsbild. Eine sporadische Färbung des Bindegewebes kann ebenfalls in Schnitten von übermäßig formalinfixierten Geweben beobachtet werden. Zur Bewertung der Färberegebnisse intakte Zellen verwenden. Nekrotische oder degenerierte Zellen werden oft unspezifisch gefärbt.<sup>3</sup> Falsch-positive Ergebnisse können aufgrund einer nichtimmunologischen Bindung von Proteinen oder Substratreaktionsprodukten beobachtet werden. In Abhängigkeit von der Art der verwendeten Immunfärbung können solche Ergebnisse auch durch endogene Enzyme wie Pseudoperoxidase (Erythrozyten), endogene Peroxidase (Zytochrom C) oder endogenes Biotin (beispielsweise Leber, Mamma, Gehirn, Niere) hervorgerufen werden. Um eine endogene Enzymaktivität bzw. eine unspezifische Enzymbindung von einer spezifischen Immunreaktivität zu unterscheiden, können zusätzliche Patientengewebe ausschließlich mit Substratchromogen bzw. mit Enzymkomplexen (Avidin-Biotin, Streptavidin, markiertes Polymer) plus Substratchromogen gefärbt werden. Falls im negativen Kontrollgewebe eine spezifische Färbung auftritt, sollten die Ergebnisse mit den Patientenproben als ungültig betrachtet werden.

### **Negative Reagenzkontrolle**

Zur Beurteilung einer unspezifischen Färbung und zur besseren Bewertung einer spezifischen Färbung an der Antigenstelle ist mit einem Schnitt jedes Patientenpräparates anstelle des primären Antikörpers eine unspezifische negative Reagenzkontrolle zu verwenden.

### **Patientengewebe**

Die mit NCL-L-ER-6F11 gefärbten Patientenproben müssen zuletzt untersucht werden. Eine positive Färbeintensität ist im Kontext einer unspezifischen Hintergrundfärbung der negativen Reagenzkontrolle zu bewerten. Wie bei jedem immunhistochemischen Test bedeutet ein negatives Ergebnis, dass das Antigen nicht nachgewiesen wurde. Ein negatives Ergebnis bedeutet jedoch nicht notwendigerweise, dass das Antigen in den getesteten Zellen / im getesteten Gewebe nicht vorlag. Bei Bedarf sollte zur Identifizierung falsch-negativer Reaktionen eine Gruppe von Antikörpern verwendet werden.

### **Erwartete Ergebnisse**

#### Normale Gewebe

Klon 6F11 erkennt das  $\alpha$ -Antigen des Östrogenrezeptors (Estrogen Receptor, ER) in den Nuklei von Zellen mit hohen ER-Spiegeln, einschließlich eines Anteils endometrialer, ovarialer und myometrialer Zellen sowie normaler duktaler Brustzellen. Die Färbung ist auch in der Mandelschleimhaut und in einem Anteil an Keimzentrum-Lymphoidzellen. (Gesamtanzahl der evaluierten normalen Fälle = 136.)

#### Tumorgewebe

Klon 6F11 färbte 137/222 der evaluierten Tumore, darunter Brusttumore (131/180), papilläre Schilddrüsenkarzinome (3/4), Eierstocktumore (2/4, einschließlich 1/2 Zystadenokarzinomen und 1/1 klarzelliger Karzinomen), metastatische Tumore unbekannter Herkunft (1/2), Lungentumore (0/4), Lebertumore (0/4), Hirntumore (0/2), Plattenepithelkarzinome des Oesophagus (0/2), Magenadenokarzinome (0/2), Weichgewebetumore (0/2), Plattenepithelkarzinome der Zunge (0/2), renale Epithelkarzinome (0/2), Plattenepithelkarzinome der Zervix (0/2), Hodenseminome (0/2), Colonadenokarzinome (0/2), rektale Adenokarzinome (0/2), Hauttumore (0/2), Plattenepithelkarzinome des Larynx (0/1) sowie atypische karzinoide Tumore des Thymus (0/1). (Gesamtanzahl der evaluierten Tumorfälle = 222).

**NCL-L-ER-6F11 wird für die Bestimmung des Östrogenrezeptor-Alphastatus beim Mammakarzinom empfohlen.**

## Allgemeine Beschränkungen

Die Immunhistochemie ist ein mehrstufiger diagnostischer Prozess, der eine spezialisierte Ausbildung auf den folgenden Gebieten erfordert: Auswahl der entsprechenden Reagenzien; Gewebeauswahl, -fixierung und -verarbeitung; Vorbereitung des IHC-Objekträgers sowie Bewertung der Färberegebnisse.

Die Gewebefärbung hängt von der Handhabung und Verarbeitung des Gewebes vor dem Färben ab. Unsachgemäßes Fixieren, Einfrieren, Auftauen, Waschen, Trocknen, Erwärmen, Schneiden oder eine Kontamination mit anderen Geweben oder Flüssigkeiten kann zu Artefakten, Antikörper-Trapping oder falsch-negativen Ergebnissen führen. Abweichende Ergebnisse können aufgrund von Unterschieden bei der Fixierung und Einbettung oder intrinsischen Unregelmäßigkeiten im Gewebe selbst entstehen.<sup>4</sup>

Eine exzessive oder unvollständige Gegenfärbung kann die korrekte Bewertung von Ergebnissen gefährden.

Die klinische Bewertung einer vorliegenden bzw. fehlenden Färbung sollte durch morphologische Studien mit entsprechenden Kontrollen ergänzt und im Kontext der Krankengeschichte des Patienten und anderer diagnostischer Tests von einem qualifizierten Pathologen vorgenommen werden.

Antikörper von Leica Biosystems Newcastle Ltd sind wo angezeigt für die Verwendung entweder auf gefrorenen oder in Paraffin eingebetteten Schnitten mit spezifischen Fixierungsanforderungen bestimmt. Es kann insbesondere bei Neoplasmen zu einer unerwarteten Antigenexpression kommen. Die klinische Bewertung eines gefärbten Gewebeschnitts muss eine morphologische Analyse und die Auswertung der entsprechenden Kontrollen einschließen.

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## Änderungen zur vorhergehenden Ausgabe

Gebrauchsempfehlungen, Erwartete Ergebnisse.

## Ausgabedatum

20 September 2012

ER-6F11-L-CE

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# Novocastra™ Anticuerpos Monoclonal Líquidos de Ratón

## Estrogen Receptor

### Código De Producto: NCL-L-ER-6F11

#### Indicaciones De Uso

*Para uso diagnóstico in vitro.*

NCL-L-ER-6F11 está indicado para la identificación cualitativa en secciones de parafina, mediante microscopía óptica, de moléculas de Estrogen Receptor. La interpretación clínica de cualquier tinción o de su ausencia debe complementarse con estudios morfológicos, con el uso de los controles adecuados, y un anatomopatólogo cualificado debe evaluarla en el contexto del historial clínico del paciente y de otras pruebas diagnósticas.

#### Principio Del Procedimiento

Las técnicas de tinción inmunohistoquímica (IHQ) permiten la visualización de antígenos mediante la aplicación secuencial de un anticuerpo específico dirigido contra el antígeno (anticuerpo primario), un anticuerpo secundario dirigido contra el anticuerpo primario y un complejo enzimático con un sustrato cromogénico, con pasos de lavado intercalados. La activación enzimática del cromógeno produce una reacción visible en el lugar en que se encuentra el antígeno. Luego se puede contrateñir la muestra y cubrirla con un cubreobjeto. Los resultados se interpretan utilizando un microscopio óptico y son de ayuda en el diagnóstico diferencial de los procesos fisiopatológicos, que pueden estar o no vinculados a un determinado antígeno.

#### Clon

6F11

#### Inmunógeno

Proteína recombinante procarciática, correspondiente a la forma alfa de longitud completa, de la molécula receptora de estrógenos humana.

#### Especificidad

Receptor de estrógenos humano.

#### Composición Del Reactivo

NCL-L-ER-6F11 es un sobrenadante de cultivo tisular líquido que contiene azida sódica como conservante.

#### Clase de Ig

IgG1

#### Concentración Total De Proteína

Total Protein

Consulte la etiqueta del vial para ver la concentración total de proteína específica del lote.

#### Concentración De Anticuerpo

Igual o superior a 67,5 mg/L según se ha determinado mediante ELISA. Consulte en la etiqueta del vial la concentración de Ig específica del lote.

#### Recomendaciones De Uso

Inmunohistoquímica con secciones de parafina.

**Recuperación de epítomos inducida por calor (HIER):** Por favor, siga las instrucciones de uso de Novocastra Epitope Retrieval Solution pH 6.

**Dilución sugerida:** 1:50 durante 30 minutos a 25 °C. Esta es tan solo una pauta y cada usuario debe determinar sus propias diluciones de trabajo óptimas.

**Visualización:** Por favor, siga las instrucciones de uso de Novolink™ Polymer Detection Systems. Para obtener más información sobre el producto o para recibir soporte, póngase en contacto con su distribuidor local o con la oficina regional de Leica Biosystems, o bien visite el sitio web de Leica Biosystems, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

El rendimiento de este anticuerpo se debe validar cuando se utiliza con otros sistemas manuales de tinción o plataformas automatizadas.

#### Almacenamiento Y Estabilidad

Almacénalo a una temperatura de 2–8 °C. No lo congele. Devuélvalo a 2–8 °C inmediatamente después de su uso. No lo utilice después de la fecha de caducidad indicada en la etiqueta del vial. Cualesquiera condiciones de almacenamiento que no sean las arriba especificadas deben ser verificadas por el usuario.

#### Preparación De Las Muestras

El fijador recomendado para secciones de tejido incluidos en parafina es formol tamponado neutro al 10%.

#### Advertencias Y Precauciones

Este reactivo se ha preparado a partir del sobrenadante de un cultivo celular. Como se trata de un producto de origen biológico, debe manipularse con precaución.

Este reactivo contiene azida sódica. Está disponible una Hoja de información sobre la seguridad del material, previa petición, o en [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Consulte las normativas nacionales, estatales, provinciales o municipales acerca de cómo desechar cualquier componente potencialmente tóxico.

Las muestras, antes y después de ser fijadas, así como todos los materiales expuestos a ellas, deben manipularse como susceptibles de transmitir una infección, y se deben desechar tomando las precauciones adecuadas.<sup>1</sup> No pipetee nunca los reactivos con la boca, y evite el contacto de la piel y de las membranas mucosas con los reactivos y las muestras. Si los reactivos o las muestras entran en contacto con zonas delicadas, lave éstas con abundante agua. Acuda inmediatamente al médico.

Reduzca al mínimo la contaminación microbiana de los reactivos; de lo contrario, podría producirse un aumento de la tinción no específica.

Cualquier tiempo o temperatura de incubación que no sean los aquí especificados pueden conducir a resultados erróneos. Cualquier cambio de tal naturaleza debe ser validado por el usuario.

### **Control De Calidad**

Las diferencias en el procesamiento de los tejidos y en los procedimientos técnicos del laboratorio del usuario pueden producir una variabilidad significativa en los resultados; por ello, es necesario que éste lleve a cabo regularmente los controles de su propio laboratorio, además de los siguientes procedimientos.

Los controles deben ser muestras frescas de autopsia, biopsia o quirúrgicas fijadas en formol, procesadas e incluidas en parafina, lo antes posible, de manera idéntica a la utilizada para la muestra o muestras del paciente o pacientes.

### **Control Tisular Positivo**

Se utiliza para indicar la preparación correcta de los tejidos y las técnicas de tinción adecuadas.

Debe incluirse un control tisular positivo por cada conjunto de condiciones de ensayo en cada tinción o serie de tinciones realizada.

Un tejido con una tinción positiva débil es más adecuado que un tejido con una tinción positiva intensa para lograr un control de calidad óptimo y para detectar niveles bajos de degradación del reactivo.<sup>2</sup>

El tejido de control positivo recomendado es endometrio.

Si el tejido de control positivo no muestra tinción positiva, los resultados de las muestras analizadas deben considerarse no válidos.

### **Control Tisular Negativo**

Debe examinarse después del control de tejido positivo, a fin de verificar la especificidad del marcado del antígeno diana por el anticuerpo primario.

El tejido de control negativo recomendado es amígdala.

O bien, la variedad de diferentes tipos de células presentes en la mayoría de los cortes de tejido ofrece con frecuencia lugares de control negativo, pero esto debe ser verificado por el usuario.

Si aparece una tinción no específica, ésta tiene generalmente aspecto difuso. En cortes de tejido fijados excesivamente con formol puede observarse también una tinción esporádica del tejido conectivo. Utilice células intactas para la interpretación de los resultados de la tinción. A menudo, las células necróticas o degeneradas quedan teñidas de forma no específica.<sup>3</sup> También pueden observarse falsos positivos causados por la unión no inmunológica a proteínas o a productos de reacción del sustrato. Estos falsos positivos pueden estar causados también por enzimas endógenas tales como la pseudoperoxidasa (eritrocitos), la peroxidasa endógena (citocromo C), o la biotina endógena (por ejemplo, de hígado, mama, cerebro, riñón), en función del tipo de inmunotinción utilizada. Para diferenciar la actividad de las enzimas endógenas o los enlaces no específicos de las enzimas de la inmunoreactividad específica, pueden teñirse otros tejidos del paciente exclusivamente con cromógeno sustrato o con complejos enzimáticos (avidina-biotina, estreptavidina, polímeros marcados) y cromógeno sustrato respectivamente. Si se produce una tinción específica del control tisular negativo, los resultados de las muestras de los pacientes deben considerarse no válidos.

### **Control De Reactivo Negativo**

Utilice un control de reactivo negativo no específico en lugar del anticuerpo primario con un corte de cada muestra del paciente a fin de evaluar la tinción no específica y obtener una mejor interpretación de la tinción específica en el lugar en que se encuentra el antígeno.

### **Tejido Del Paciente**

Examine las muestras del paciente o pacientes teñidas con NCL-L-ER-6F11 al final. La intensidad de la tinción positiva debe valorarse en el contexto de cualquier tinción de fondo no específica del control de reactivo negativo. Como con cualquier prueba inmunohistoquímica, un resultado negativo significa que no se ha detectado antígeno, y no que el antígeno esté ausente en las células o tejido probados. Si es necesario, use un panel de anticuerpos para identificar falsas reacciones negativas.

### **Resultados esperados**

#### Tejidos normales

El clon 6F11 detecta el antígeno del receptor estrogénico (RE) alfa de los núcleos de células que expresan altos niveles de RE, incluida una proporción de células endometriales, ováricas y miometriales y células ductales de mama normales. También se puede apreciar tinción en la mucosa de la tonsila y en una proporción de células linfoides con centros germinales. (Número total de casos normales evaluados = 136).

#### Tejidos tumorales

El clon 6F11 tiñó 137 de los 222 tumores evaluados, incluidos tumores de mama (131/180), carcinomas papilares tiroideos (3/4), tumores de ovario (2/4), incluidos 1/2 cistoadenocarcinomas y 1/1 carcinomas con células claras), tumores metastásicos de origen desconocido (1/2), tumores pulmonares (0/4), tumores hepáticos (0/4), tumores cerebrales (0/2), carcinomas de células escamosas del esófago (0/2), adenocarcinomas de estómago (0/2), tumores del tejido blando (0/2), carcinomas de células escamosas de la lengua (0/2), carcinomas de células renales (0/2), carcinomas de células escamosas del cuello del útero (0/2), seminomas testiculares (0/2), adenocarcinomas de colon (0/2), adenocarcinomas rectales (0/2), tumores cutáneos (0/2), carcinomas de células escamosas de la laringe (0/1) y carcinoides atípicos de timo (0/1). (Número total de casos de tumores evaluados = 222).

**NCL-L-ER-6F11 se recomienda para determinar el estado del receptor de estrógenos alfa del tejido de cáncer de mama.**



## Limitaciones Generales

La inmunohistoquímica es un proceso de diagnóstico en varias fases que abarca: la formación especializada en la selección de los reactivos apropiados, la selección, fijación y procesamiento de tejidos, la preparación del portaobjeto para IHQ, y la interpretación de los resultados de la tinción.

La tinción de los tejidos depende de la manipulación y el procesamiento del tejido previos a la tinción. Una fijación, congelación, descongelación, lavado, secado, calentamiento o seccionamiento incorrectos, o la contaminación con otros tejidos o líquidos pueden generar artefactos, atrapamiento del anticuerpo o falsos negativos. La aparición de resultados incoherentes puede deberse a variaciones en los métodos de fijación y de inclusión, o a irregularidades inherentes al tejido.<sup>4</sup>

Una contratinción excesiva o incompleta puede poner en peligro la interpretación correcta de los resultados.

La interpretación clínica de cualquier tinción o de su ausencia debe complementarse con estudios morfológicos, con el uso de los controles adecuados, y un anatomopatólogo cualificado debe evaluarla en el contexto del historial clínico del paciente y de otras pruebas diagnósticas.

Los anticuerpos de Leica Biosystems Newcastle Ltd son para utilizarlos, según se indique, con secciones congeladas o incluidas en parafina, con requisitos de fijación específicos. Puede producirse una expresión inesperada del antígeno, especialmente en las neoplasias. La interpretación clínica de cualquier sección de tejido teñida debe incluir un análisis morfológico y la evaluación de los controles apropiados.

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## Correcciones A La Publicación Anterior

Recomendaciones De Uso, Resultados esperados.

## Fecha De Publicación

20 de septiembre de 2012

# Novocastra™ Anticorpo Monoclonal Líquido de Ratinho

## Estrogen Receptor

### Código Do Produto: NCL-L-ER-6F11

#### Utilização prevista

*Para utilização em diagnósticos in vitro.*

NCL-L-ER-6F11 foi concebido para efectuar a identificação qualitativa da moléculas de Estrogen Receptor por microscopia óptica, em secções parafinizadas. A interpretação clínica de qualquer coloração ou da sua ausência deve ser complementada por estudos morfológicos empregando os devidos controlos e deve ser avaliada por um patologista qualificado, dentro do contexto do historial clínico do doente e de outros testes de diagnóstico.

#### Princípio Do Procedimento

As técnicas de coloração imunohistoquímica (IHC) permitem que se faça a visualização de antígenos por meio da aplicação sequencial de um anticorpo específico do antígeno (o anticorpo primário), de um anticorpo secundário ao anticorpo primário, e de um complexo enzimático com um substrato cromogénico e etapas de lavagem de permeio. A activação enzimática do cromogénio resulta num produto de reacção visível no local do antígeno. A amostra pode então ser contrastada e coberta com uma lamela. Os resultados são interpretados por meio de um microscópio óptico, e ajudam a formular o diagnóstico diferencial dos processos fisiopatológicos, os quais podem ou não estar associados a antígenos específicos.

#### Clone

6F11

#### Imunogénio

Proteína recombinante procarciótica correspondendo à forma alfa de comprimento total da molécula receptora de estrogénio humana.

#### Especificidade

Receptor de estrogénio humano.

#### Composição Do Reagente

NCL-L-ER-6F11 é o sobrenadante líquido da cultura de um tecido contendo de azida de sódio como produto conservante.

#### Classe De Ig

IgG1

#### Concentração Total De Proteína

Consultar a etiqueta da ampola para determinar a concentração total de proteína do lote específico.

#### Concentração De Anticorpo

Maior que ou igual a 67,5 mg/L, conforme determinado por ELISA. Consultar a etiqueta da ampola para determinar a concentração de Ig do lote específico.

#### Recomendações Sobre A Utilização

Imunohistoquímica em cortes de inclusões em parafina.

**Recuperação de epitopos induzida pelo calor (HIER):** Queira seguir as instruções de utilização de Novocastra Epitope Retrieval Solution pH 6.

**Diluição sugerida:** 1:50 durante 30 minutos a 25 °C. Esta recomendação serve apenas de orientação e os utilizadores devem determinar as suas diluições óptimas de trabalho.

**Visualização:** Queira seguir as instruções de utilização de Novolink™ Polymer Detection Systems. Para informação adicional do produto ou assistência, contactar o seu distribuidor local ou escritório regional de Leica Biosystems ou, alternativamente, visitar o site web de Leica Biosystems, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

O desempenho deste anticorpo deve ser validado quando utilizado com outros sistemas manuais de coloração ou plataformas automáticas.

#### Armazenamento E Estabilidade

Armazenar a 2–8 °C. Não congelar. Retornar à temperatura de 2–8 °C imediatamente após a utilização. Não utilizar após o prazo de validade indicado no rótulo do recipiente. As condições de armazenamento que diferirem das que se encontram especificadas acima devem ser verificadas pelo utilizador.

#### Preparação Das Amostras

O fixador recomendado é formal tamponado neutro a 10% para secções de tecido envolvidas em parafina.

#### Avisos E Precauções

Este reagente foi preparado a partir do sobrenadante de cultura celular. Visto ser um produto biológico, deve ser manuseado com o devido cuidado.

Este reagente contém azida sódica. Encontra-se disponível uma Ficha de Dados de Segurança do Material, mediante pedido ou através do site [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Consultar a legislação aplicável em relação ao descarte de quaisquer componentes potencialmente tóxicos.

As amostras, antes e depois da sua fixação, bem como todos os materiais expostos às mesmas, devem ser manipulados como se tivessem a capacidade de transmitir infecções e devem ser descartados com as devidas precauções.<sup>1</sup> Não pipetar nunca os reagentes com a boca e evitar o contacto entre a pele e membranas mucosas e os reagentes e amostras. Caso os reagentes ou amostras entrem em contacto com áreas sensíveis, lavar com grandes quantidades de água. Consultar um médico.

Minimizar a contaminação microbiana dos reagentes para evitar a possibilidade do aumento da coloração não específica.

Os períodos de incubação ou temperaturas diferentes dos que foram especificados poderão dar azo a resultados errados. Todas as alterações desse tipo devem ser validadas pelo utilizador.

## Controlo Da Qualidade

As diferenças entre os diferentes métodos e técnicas de processamento de tecidos no laboratório do utilizador podem causar uma grande variabilidade de resultados, requerendo a realização frequente de controlos internos suplementares aos procedimentos que se seguem.

Os controlos devem ser amostras de autópsia/biopsia/cirurgia frescas, fixadas em formol, processadas e envolvidas em cera parafínica logo que possível, da mesma maneira que a(s) amostra(s) do(s) doente(s).

## Controlo De Tecido Positivo

Usado para assinalar os tecidos correctamente preparados e as técnicas de coloração indicadas.

Cada conjunto de condições de testes, em cada processo de coloração, deve incluir um controlo de tecido positivo.

Os tecidos com uma coloração positiva fraca são mais indicados do que os têm uma coloração positiva forte para proporcionarem um controlo de qualidade óptimo, bem como para detectar níveis reduzidos de degradação dos reagentes.<sup>2</sup>

O tecido de controlo positivo recomendado é o endométrio.

Se o controlo de tecido positivo não demonstrar uma coloração positiva, os resultados obtidos com as amostras de testes devem ser considerados inválidos.

## Controlo De Tecido Negativo

Este deve ser examinado depois do controlo de tecido positivo para verificar a especificidade da marcação do antígeno objectivado pelo anticorpo primário.

O controlo de tecido negativo recomendado é a amígdala.

Alternativamente, a variedade de diferentes tipos de células presentes na maioria das secções de tecidos oferece muitas vezes locais de controlo negativo, mas isto deve ser verificado pelo utilizador.

A coloração não específica, caso ocorra, tem geralmente um aspecto difuso. A coloração esporádica do tecido conjuntivo pode também ter lugar em secções de tecido excessivamente fixado em formol. Devem utilizar-se células intactas para a interpretação dos resultados da coloração. As células necróticas ou degeneradas causam muitas vezes uma coloração não específica.<sup>3</sup> Podem verificar-se resultados positivos falsos devido à ligação não imunológica de proteínas ou de produtos da reacção do substrato. Esses resultados podem também ser causados por enzimas endógenas tais como a pseudoperoxidase (eritrócitos), a peroxidase endógena (citocromo C), ou a biotina endógena (ex. no fígado, mama, cérebro ou rim) dependendo do tipo de imunocoloração utilizado. Para diferenciar entre a actividade das enzimas endógenas e as ligações não específicas de enzimas de imunoreactividade específica, podem colorir-se tecidos adicionais dos doentes exclusivamente com substrato cromogénio ou com complexos de enzimas (avidina-biotina, estreptavidina, polímero marcado) e substrato-cromogénio, respectivamente. Se ocorrer a coloração específica no controlo de tecido negativo, os resultados dos testes feitos com as amostras do doente devem ser considerados inválidos.

## Controlo De Reagente Negativo

Utilizar um controlo de reagente negativo não específico em vez do anticorpo primário com uma secção de cada amostra de doente para avaliar a coloração não específica e permitir uma melhor interpretação da coloração específica no local do antígeno.

## Tecido Do Doente

Examinar as amostras do doente coloridas com NCL-L-ER-6F11 em último lugar. A intensidade da coloração positiva deve ser avaliada dentro do contexto de qualquer coloração não específica de fundo do controlo de reagente negativo. Tal como com qualquer teste imunohistoquímico, um resultado negativo significa que o antígeno não foi detectado, e não que o antígeno se encontrava ausente das células ou tecido analisados. Se necessário, deve utilizar-se um painel de anticorpos para identificar reacções falso-negativas.

## Resultados Previstos

### Tecidos normais

O clone 6F11 deteta o antígeno alfa de recetor de estrogénio (ER) no núcleo de células que expressam níveis elevados de ER, incluindo uma proporção de células endometriais, ováricas e miometriais, e células ductais mamárias normais. A coloração também pode ser observada na mucosa das amígdalas e numa proporção das células linfóides centrais germinais. (Número total de casos normais avaliados = 136).

### Tecidos tumorais

O clone 6F11 corou 137 dos 222 tumores avaliados, incluindo tumores mamários (131/180), carcinomas papilares da tiróide (3/4), tumores ováricos (2/4), incluindo 1/2 citadenocarcinomas e 1/1 carcinomas de células claras), tumores metastáticos de origem desconhecida (1/2), tumores pulmonares (0/4), tumores hepáticos (0/4), tumores cerebrais (0/2), carcinomas de células escamosas do esófago (0/2), adenocarcinomas do estômago (0/2), tumores dos tecidos moles (0/2), carcinomas de células escamosas da língua (0/2), carcinomas de células renais (0/2), carcinomas de células escamosas do colo do útero (0/2), seminomas testiculares (0/2), adenocarcinomas do cólon (0/2), adenocarcinomas retais (0/2), tumores de pele (0/2), carcinomas de células escamosas da laringe (0/1) e tumores carcinóides atípicos do timo (0/1). (Número total de casos de tumores avaliados = 222).

**NCL-L-ER-6F11 é recomendado para determinação do estado do alfa receptor de estrogénio do tecido do cancro da mama.**

## Limitações Gerais

A imunohistoquímica é um processo diagnóstico em múltiplas etapas que consta de: uma formação especializada na selecção dos reagentes apropriados, selecção, fixação e processamento de tecidos, preparação das lâminas de IHQ e interpretação dos resultados das colorações.

A coloração de tecidos depende do seu manuseamento e processamento antes da sua coloração. A fixação, congelação, descongelação, lavagem, secagem, aquecimento ou corte incorrectos das amostras, ou a sua contaminação com outros tecidos ou fluidos, podem produzir artefactos, retenção de anticorpos, ou resultados falso-negativos. Os resultados inconsistentes podem dever-se a variações nos métodos de fixação e envolvimento ou a irregularidades inerentes ao tecido.<sup>4</sup>

Uma contrastação excessiva ou incompleta pode comprometer a correcta interpretação dos resultados.

A interpretação clínica de qualquer coloração ou da sua ausência deve ser complementada por estudos morfológicos empregando os devidos controlos e deve ser avaliada por um patologista qualificado, dentro do contexto do historial clínico do doente e de outros testes de diagnóstico.

Os anticorpos da Leica Biosystems Newcastle Ltd destinam-se a serem utilizados, conforme indicado, em secções de tecido ou congeladas ou envolvidas em parafina, com requisitos de fixação específicos. Pode ocorrer uma expressão inesperada de antígeno, especialmente em neoplasmas. A interpretação clínica de qualquer secção de tecido colorido deverá incluir a análise morfológica e a avaliação de controlos apropriados.

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## **Emendas Da Edição Anterior**

Recomendações Sobre A Utilização, Resultados Previstos.

## **Data De Emissão**

20 de Setembro de 2012

# Novocastra™ Flytande Monoklonal Musantikropp

## Estrogen Receptor

### Produktkod: NCL-L-ER-6F11

#### **Avsedd Användning**

*För in vitro diagnostisk användning.*

NCL-L-ER-6F11 är avsedd för kvalitativ identifiering med ljusmikroskopi av Estrogen Receptor-molekyler i paraffinsnitt. Den kliniska tolkningen av all färgning eller dess frånvaro bör kompletteras med morfologiska undersökningar som använder korrekta kontroller och utvärderas av kvalificerad patalog inom ramen för patientens kliniska anamnes och andra diagnostiska tester.

#### **Metodens Princip**

Immunhistokemiska (IHC) färgningstekniker tillåter visualisering av antigener genom sekvenstillämpning av en specifik antikropp till antigenet (primär antikropp), en sekundär antikropp till den primära antikroppen och ett enzymkomplex med ett kromogent substrat med inlagda tvättsteg. Den enzymatiska aktiveringen av kromogenet resulterar i en synlig reaktionsprodukt på antigenområdet. Proverna kan då kontrastfärgas och förses med täckglas. Resultaten tolkas med ljusmikroskop och bidrar till differentialdiagnosen av patofysiologiska processer som eventuellt kan associeras till ett särskilt antigen.

#### **Klon**

6F11

#### **Immunogen**

Prokaryotiskt rekombinant protein motsvarande den fulla längden alfaformen av den humana östrogenreceptormolekylen.

#### **Specifitet**

Human östrogenreceptor.

#### **Reagensinnehåll**

NCL-L-ER-6F11 är en flytande supernatant från vävnadsodling som innehåller natriumazid som konserveringsmedel.

#### **Ig-klass**

IgG1

#### **Total Proteinkoncentration** Total Protein

Se flaskans etikett för total specifik proteinkoncentration för satsen.

#### **Antikropps-koncentration**

Större än eller lika med 67,5 mg/l fastställt genom ELISA. Se flaskans etikett för specifik Ig-koncentration för satsen.

#### **Rekommendationer Vid Användning**

Immunhistokemi på paraffinsnitt.

**Värmeinducerad epitopåtervinning (HIER):** Vänligen följ instruktionerna för användning i Novocastra Epitope Retrieval Solution pH 6.

**Föreslagen spädning:** 1:50 i 30 minuter vid 25 °C. Detta är endast en riktlinje och användare bör själva fastställa den optimala bruksspädningen.

**Visualisering:** Vänligen följ instruktionerna för användning i Novolink™ Polymer Detection Systems. Om ytterligare produktinformation eller stöd behövs, kontakta då din lokala distributör eller Leica Biosystems regionalkontor, alternativt in på Leica Biosystems webbplats, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Denna antikropps prestanda ska valideras när den används med andra manuella infärgningssystem eller automatiserade plattformar.

#### **Förvaring Och Stabilitet**

Förvara vid 2–8 °C. Frys ej. Återgå till 2–8 °C direkt efter användning. Använd ej efter det utgångsdatum som anges på flaskans etikett. Förvaringsförhållanden som skiljer sig från de ovannämnda måste kontrolleras av användaren.

#### **Preparation Av Prov**

Rekommenderat fixeringsmedel för paraffinbäddade vävnadssnitt är 10% neutralbuffrat formalin.

#### **Varningar Och Försiktighetsåtgärder**

Reagenset har förberetts från supernatanten av vävnadsodlingar. Eftersom det är en biologisk produkt bör skälighets försiktighet iaktas vid hantering.

Detta reagens innehåller natriumazid. Materialsäkerhetsdatablad finns att få på begäran eller från [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

För kassering av potentiellt toxiska komponenter hänvisas till nationella eller lokala bestämmelser.

Före och efter fixering bör prover och alla material som har varit utsatta för dem hanteras som om det finns risk för att de kan överföra infektioner och kasseras med iakttagande av försiktighet. Pipettera aldrig reagenser med munnen och se till att huden och slemhinnorna inte kommer i kontakt med reagens och prover. Om reagens eller prover kommer i kontakt med känsliga områden, tvätta med rikliga mängder vatten. Rådgör med läkare.

Minimera mikrobisk kontaminering av reagens annars kan en ökning av ospecifik färgning ske.

Inkubationstider eller temperaturer som skiljer sig från dem som specificeras kan ge felaktiga resultat. Alla sådana förändringar måste kontrolleras av användaren.

## Kvalitetskontroll

Skilnader i vävnadsbehandling och tekniska metoder i användarens laboratorium kan ge stor variation i resultaten vilket kan göra det nödvändigt att genomföra regelbundna interna kontroller utöver följande metoder.

Kontroller bör vara färskas obduktions-/biopsi-/kirurgi prover som snarast möjligt formalinfixeras, bearbetas och paraffinbäddas på samma sätt som patientprover.

## Positiv Vävnadskontroll

Används för att ange korrekt förberedda vävnader och rätt färgningstekniker.

En positiv vävnadskontroll bör ingå i varje uppsättning av testförhållanden vid varje färgningskörning.

En vävnad med svag positiv färgning är mer lämplig för optimal kvalitetskontroll och för att upptäcka låga nivåer av reagensdegradering än en vävnad med stark positiv färgning.<sup>2</sup>

Endometrium rekommenderas som positiv kontrollvävnad.

Om den positiva vävnadskontrollen inte uppvisar positiv färgning bör resultat med testproverna anses vara ogiltiga.

## Negativ Vävnadskontroll

Bör undersökas efter den positiva vävnadskontrollen för att fastställa specificiteten för märkningen av målantigenet med den primära antikroppen.

Tonsill rekommenderades som negativ kontrollvävnad.

Alternativt ger ofta en mängd olika celltyper som finns i de flesta vävnadssnitt negativa kontrollområden men detta bör kontrolleras av användaren.

Ospecifik färgning, om det förekommer, har ofta ett diffust utseende. Sporadisk färgning av bindväv kan också observeras i snitt från överflödigt formalinfixerade vävnader. Använd intakta celler för tolkning av färgningsresultat. Nekrotiska eller degenererade celler fångar ofta ospecifikt.<sup>3</sup> Falskt positiva resultat kan uppstå p.g.a. icke-immunologisk bindning av proteiner eller substratreaktionsprodukter. De kan också orsakas av endogena enzymer som pseudoperoxidas (erythrocyter), endogent peroxid (cytokrom C) eller endogent biotin (t.ex. lever, bröst, hjärna, njure) beroende på typ av immunfärgning som används. För att skilja endogen enzymaktivitet eller ospecifik enzymbindning från specifik immunreaktivitet kan ytterligare patientvävnader färgas exklusivt med respektive substratkromogen eller enzymkomplex (avidin-biotin, streptavidin, märkt polymer) och substrat-kromogen. Om specifik färgning sker i den negativa vävnadskontrollen bör resultat med patientprover anses vara ogiltiga.

## Negativ Reagenskontroll

Använd en ospecifik negativ reagenskontroll istället för den primära antikroppen med ett snitt från varje patientprov för att utvärdera ospecifik färgning och tillåta bättre tolkning av specifik färgning på antigenområdet.

## Patientvävnad

Undersök patientprover färgade med NCL-L-ER-6F11 sist. Positiv färgningsintensitet bör utvärderas inom ramen för all ospecifik bakgrunds-färgning av den negativa reagenskontrollen. Som vid alla immunhistokemiska tester betyder ett negativt resultat att antigenet inte upptäcktes och inte att det inte förekom i de analyserade cellerna/vävnaderna. Använd vid behov en antikroppspanel för att identifiera falskt negativa reaktioner.

## Förväntade Resultat

### Normal vävnad

Klonen 6F11 detekterar östrogenreceptorn (ER) alfa-antigen i kärnor hos celler som uttrycker höga nivåer av ER, inklusive en del av endometrial-, ovarial- och myometrialceller och normala bröstduktala celler. Färgning kan också ses i slemhinna från tonsill och en del av germinalcentrats lymfceller. (Totalt antal normala fall som utvärderats = 136).

### Tumörvävnader

Klonen 6F11 färgade 137/222 utvärderade tumörer, däribland brösttumörer (131/180), papillära carcinom från sköldkörtel (3/4), ovariala tumörer (2/4, inklusive 1/2 cystadenocarcinom och 1/1 klarcellskarcinom), metastatiska tumörer av okänt ursprung (1/2), lungtumörer (0/4), levertumörer (0/4), hjärntumörer (0/2), skivpitelskarcinom från matstrupe (0/2), adenocarcinom från magsäck (0/2), tumörer från mjuk vävnad (0/2), skivcellskarcinom från tunga (0/2), njurcellskarcinom (0/2), skivpitelskarcinom från livmoderhals (0/2), testikelseminom (0/2), kolon-adenocarcinom (0/2), rektala adenocarcinom (0/2), hudtumörer (0/2), skivpitelskarcinom från struphuvud (0/1) och atypiska karcinoida tumörer från thymus (0/1). (Totalt antal fall av tumörer som utvärderats = 222).

**NCL-L-ER-6F11 rekommenderas för att fastställa östrogenreceptorernas alfa-status av bröstcancervävnad.**

## Allmänna Begränsningar

Immunhistokemi är en diagnostisk process i flera steg som kräver specialiserad utbildning i urvalet av lämpliga reagens, val av vävnad, fixering och bearbetning, förberedelse av IHC-objektglaset samt tolkning av färgningsresultaten.

Vävnadsfärgning påverkas av hantering och bearbetning av vävnaden före färgningen. Felaktig fixering, nedfrysning, upptining, tvättning, torkning, uppvärmning, snittning eller kontaminering av andra vävnader eller vätskor kan framställa artefakter, infångande av antikroppar eller falskt negativa resultat. Motsägelsefulla resultat kan bero på variationer av fixerings- och inbäddningsmetoder eller på naturliga oregelbundenheter i vävnaden.<sup>4</sup>

Överflödigt eller ofullständig kontrastfärgning kan försvåra en korrekt tolkning av resultatet.

Den kliniska tolkningen av all färgning eller dess frånvaro bör kompletteras med morfologiska undersökningar som använder korrekta kontroller och utvärderas av kvalificerad patolog inom ramen för patientens kliniska anamnes och andra diagnostiska tester.

Antikroppar från Leica Biosystems Newcastle Ltd är till för användning så som anges på antingen frysta eller paraffinbäddade snitt med specifika fixeringskrav. Övrigt antigenuttryck kan ske, speciellt i neoplasmer. Morfologisk analys och utvärdering av lämpliga kontroller måste ingå i den kliniska tolkningen av alla färgade vävnadssnitt.

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## Rättelser Av Tidigare Utgivning

Rekommendationer Vid Användning, Förväntade Resultat.

## Utgivningsdatum

20 september 2012

# Novocastra™ Υγρό μονοκλωνικό αντίσωμα ποντικού

## Estrogen Receptor

### Κωδικός είδους: NCL-L-ER-6F11

#### Χρήση Για Την Οποία Προορίζεται

*Gia in vitro διαγνωστική χρήση.*

Το NCL-L-ER-6F11 προορίζεται για την ποιοτική ταυτοποίηση με μικροσκοπία φωτός της ανθρωπίνης Μόρια Estrogen Receptor σε τομές παραφίνης. Η κλινική ερμηνεία οποιασδήποτε χρώσης ή της απουσίας της θα πρέπει να συμπληρώνεται με μορφολογικές μελέτες που χρησιμοποιούν σωστούς μάρτυρες και θα πρέπει να αξιολογείται στα πλαίσια του κλινικού ιστορικού του ασθενούς και άλλων διαγνωστικών εξετάσεων από ειδικευμένο παθολογοανατόμο.

#### Αρχή Της Διαδικασίας

Οι τεχνικές ανοσοϊστοχημικής (IHC) χρώσης επιτρέπουν την οπτικοποίηση των αντιγόνων μέσω της διαδοχικής εφαρμογής ενός ειδικού αντισώματος στο αντιγόνο (πρωτοταγές αντίσωμα), ενός δευτεροταγούς αντισώματος στο πρωτοταγές αντίσωμα και ενός ενζυμικού συμπλόκου με χρωμογόνο υπόστρωμα με παρεμβαλλόμενα βήματα πλύσης. Η ενζυμική ενεργοποίηση του χρωμογόνου έχει ως αποτέλεσμα το σχηματισμό ενός ορατού προϊόντος αντίδρασης στη θέση του αντιγόνου. Το δείγμα μπορεί κατόπιν να υποβληθεί σε αντίχρωση και να καλυφθεί με καλυπτήρια. Τα αποτελέσματα ερμηνεύονται με χρήση μικροσκοπίου φωτός και βοηθούν στη διαφορική διάγνωση παθολογικών εξεργασιών, οι οποίες ενδέχεται ή όχι να σχετίζονται με ένα συγκεκριμένο αντιγόνο.

#### Κλώνος

6F11

#### Ανοσογόνο

Προκαρκατωτική ανασυνδυασμένη πρωτεΐνη που αντιστοιχεί στην α-μορφή πλήρους μήκους του μορίου του ανθρώπινου υποδοχέα οιστρογόνων.

#### Ειδικότητα

Ανθρώπινος υποδοχέας οιστρογόνων.

#### Σύνθεση Αντιδραστήριου

Το NCL-L-ER-6F11 είναι ένα υγρό υπερκείμενο ιστοκαλλιέργειας που περιέχει αζίδιο του νατρίου ως συντηρητικό.

#### Τάξη Ig

IgG1

#### Ολική Συγκέντρωση Πρωτεΐνης

Total Protein

Για την ολική συγκέντρωση πρωτεΐνης που είναι ειδική για την εκάστοτε παρτίδα, ανατρέξτε στην ετικέτα του φιαλιδίου.

#### Συγκέντρωση Αντισώματος

Μεγαλύτερη ή ίση με 67,5 mg/L, όπως προσδιορίζεται με ELISA. Για τη συγκέντρωση Ig που είναι ειδική για την εκάστοτε παρτίδα, ανατρέξτε στην ετικέτα του φιαλιδίου.

#### Συστάσεις Για Τη Χρήση

Ανοσοϊστοχημεία σε παρασκευάσματα παραφίνης.

**Ανάκτηση Επίτοπου με Θερμική Επαγωγή (HIER):** Παρακαλούμε ακολουθήστε τις οδηγίες για τη χρήση στο Novocastra Epitope Retrieval Solution pH 6.

**Προτεινόμενη διάλυση:** 1:50 επί 30 λεπτά σε 25 °C. Παρέχεται ως οδηγός και οι χρήστες θα πρέπει να καθορίζουν τις δικές τους διαλύσεις εργασίας.

**Απεικόνιση:** Παρακαλούμε ακολουθήστε τις οδηγίες χρήσης στο Novolink™ Polymer Detection Systems. Για περισσότερες πληροφορίες για το προϊόν ή για υποστήριξη, επικοινωνήστε με τον τοπικό διανομέα ή το περιφερειακό γραφείο της Leica Biosystems ή εναλλακτικά επισκεφθείτε τον ιστότοπο της Leica Biosystems, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Η απόδοση του συγκεκριμένου αντισώματος θα πρέπει να επικυρωθεί όταν χρησιμοποιηθεί μαζί με άλλα μη αυτόματα συστήματα χρώσης ή αυτοαποποιημένες πλατφόρμες.

#### Φύλαξη Και Σταθερότητα

Φυλάσσετε στους 2–8 °C. Μην καταψύχετε. Επαναφέρετε στους 2–8 °C αμέσως μετά τη χρήση. Μην χρησιμοποιείτε μετά την ημερομηνία λήξης που αναγράφεται στην ετικέτα του φιαλιδίου. Τυχόν συνθήκες φύλαξης διαφορετικές από εκείνες που καθορίζονται παραπάνω πρέπει να επαληθεύονται από το χρήστη.

#### Παρασκευή Δείγματος

Το συνιστώμενο μονιμοποιητικό είναι ουδέτερο ρυθμιστικό διάλυμα φορμόλης 10% για τομές ιστού εγκλεισμένες σε παραφίνη.

#### Προειδοποιήσεις Και Προφυλάξεις

Το αντιδραστήριο αυτό έχει παρασκευαστεί από το υπερκείμενο κυτταροκαλλιέργειας. Επειδή είναι βιολογικό προϊόν, θα πρέπει να δίνεται εύλογη προσοχή κατά το χειρισμό του.

Αυτό το αντιδραστήριο περιέχει αζίδιο του νατρίου. Δελτίο δεδομένων ασφαλείας υλικού διατίθεται κατόπιν αιτήματος ή από τη διεύθυνση [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Συμβουλευτείτε τους ομοσπονδιακούς, πολιτειακούς ή τοπικούς κανονισμούς για απόρριψη τυχόν δυνητικών τοξικών συστατικών.

Ο χειρισμός δειγμάτων, πριν και μετά τη μονιμοποίηση, καθώς και όλων των υλικών που έχουν εκτεθεί σε αυτά, θα πρέπει να γίνεται ως εάν ήταν δυνητικά μετάδοσης λοίμωξης και η απόρριψή τους να πραγματοποιείται λαμβάνοντας τις σωστές προφυλάξεις. Μην αναρροφάτε ποτέ με πιπέτα αντιδραστήρια με το στόμα και αποφυγετε την επαφή του δέρματος και των βλεννογόνων με αντιδραστήρια και δείγματα. Εάν τα αντιδραστήρια ή τα δείγματα έλθουν σε επαφή με ευαίσθητες περιοχές, πλύνετε με άφθονες ποσότητες νερού. Ζητήστε τη συμβουλή ιατρού.



Ελαχιστοποιήστε τη μικροβιακή μόλυνση των αντιδραστηρίων, διότι ενδέχεται να συμβεί αύξηση μη ειδικής χρώσης. Χρόνοι ή θερμοκρασίες επώασης διαφορετικές από εκείνες που καθορίζονται ενδέχεται να δώσουν εσφαλμένα αποτελέσματα. Τυχόν τέτοιες μεταβολές πρέπει να επικυρώνονται από το χρήστη.

## Ποιοτικός Έλεγχος

Τυχόν διαφορές στην επεξεργασία των ιστών και τις τεχνικές διαδικασίες στο εργαστήριο του χρήστη ενδέχεται να προκαλέσουν σημαντική μεταβλητότητα στα αποτελέσματα, καθιστώντας αναγκαία την τακτική εκτέλεση εσωτερικών ελέγχων επιπλέον των ακόλουθων διαδικασιών.

Οι μάρτυρες θα πρέπει να είναι φρέσκα δείγματα νεκροψιάς/βιοψιάς/χειρουργικά δείγματα, τα οποία είναι μονιμοποιημένα σε φορμόλη, επεξεργασμένα και εγκλεισμένα σε κηρό παραφίνης, το συντομότερο δυνατό με τον ίδιο τρόπο με το(α) δείγμα(τα) του ασθενούς.

## Θετικός Μάρτυρας Ιστού

Χρησιμοποιείται για να υποδεικνύει σωστά παρασκευασμένους ιστούς και σωστές τεχνικές χρώσης.

Θα πρέπει να περιλαμβάνεται ένας θετικός μάρτυρας ιστού για κάθε σύνολο συνθηκών εξέτασης σε κάθε εκτέλεση χρώσης.

Ένας ιστός με ασθενή θετική χρώση είναι πιο κατάλληλος από έναν ιστό με ισχυρή θετική χρώση για βέλτιστο έλεγχο ποιότητας και για την ανίχνευση τοπικών μικρών επιπέδων τυχόν αποδόμησης των αντιδραστηρίων.<sup>2</sup>

Συνιστώμενος ιστός θετικού μάρτυρα είναι το ενδομήτριο.

Εάν ο θετικός μάρτυρας ιστού δεν παρουσιάζει θετική χρώση, τα αποτελέσματα με τα δείγματα της εξέτασης θα πρέπει να θεωρούνται άκυρα.

## Αρνητικός Μάρτυρας Ιστού

Θα πρέπει να εξετάζεται μετά τον θετικό μάρτυρα ιστού για την επαλήθευση της ειδικότητας της επίσημης της ανιχνύου-στόχου από το πρωτοπαγές αντίσωμα.

Συνιστώμενος ιστός αρνητικού μάρτυρα είναι η αμυγδαλή.

Εναλλακτικά, η ποικιλία διαφόρων κυτταρικών τύπων που υπάρχουν στις περισσότερες τομές ιστών παρέχει συχνά θέσεις αρνητικού μάρτυρα, αλλά αυτό πρέπει να επαληθεύεται από το χρήστη.

Μη ειδική χρώση, εάν υπάρχει, έχει συνήθως διάχυτη εμφάνιση. Ενδέχεται επίσης να παρατηρηθεί αποραδική χρώση του συνδετικού ιστού σε τομές από ιστούς που έχουν μονιμοποιηθεί με υπερβολική ποσότητα φορμόλης. Χρησιμοποιείτε άθικτα κύτταρα για την ερμηνεία των αποτελεσμάτων της χρώσης. Νεκρωτικά ή εκφυλισμένα κύτταρα παρουσιάζουν συχνά μη ειδική χρώση.<sup>3</sup> Ενδέχεται να παρατηρηθούν ψευδώς θετικά αποτελέσματα λόγω μη ανοσολογικής δέσμευσης των πρωτεϊνών ή των προϊόντων αντίδρασης του υποστρώματος. Ενδέχεται επίσης να προκληθούν από ενδογενή ένζυμα, όπως η ψευδοπυροξειδάση (ερυθροκύτταρα), η ενδογενής υπεροξειδάση (κυτόχρωμα C) ή η ενδογενής βιοτίνη (π.χ. ήπαρ, μαστός, εγκέφαλος, νεφρός) ανάλογα με τον τύπο ανοσοχρώσης που χρησιμοποιείται. Για τη διαφοροποίηση της ενδογενούς ενζυμικής δραστηριότητας ή της μη ειδικής δέσμευσης των ενζύμων από ειδική ανοσοαντιδραστικότητα, είναι δυνατό να χρωματιστούν αποκλειστικά επιπλέον ιστού ασθενών με χρωμογόνο υποστρώματος ή ενζυμικά σύμπλοκα (αβιδίνη-βιοτίνη, στρεπταβιδίνη, σημιασμένο πολυμερές) και υπόστρωμα-χρωμογόνο, αντίστοιχα. Εάν παρουσιαστεί κοινή χρώση στον αρνητικό μάρτυρα ιστού, τα αποτελέσματα με τα δείγματα ασθενούς θα πρέπει να θεωρούνται άκυρα.

## Αρνητικός Μάρτυρας Αντιδραστηρίου

Χρησιμοποιείτε έναν μη ειδικό αρνητικό μάρτυρα αντιδραστηρίου αντί του πρωτοπαγούς αντισώματος με μια τομή κάθε δείγματος ασθενούς για την αξιολόγηση μη ειδικής χρώσης και για να επιτρέπεται καλύτερη ερμηνεία της ειδικής χρώσης στη θέση του ανιχνύου.

## Ιστός Ασθενούς

Εξετάστε τελευταία τα δείγματα ασθενούς που έχουν χρωματιστεί με το NCL-L-ER-6F11. Η ένταση της θετικής χρώσης θα πρέπει να εκτιμάται στα πλαίσια τυχόν μη ειδικής χρώσης υποβάθρου του αρνητικού μάρτυρα αντιδραστηρίου. Όπως συμβαίνει με οποιαδήποτε ανοσοϊστοχημική εξέταση, ένα αρνητικό αποτέλεσμα σημαίνει ότι το ανιγόνο δεν ανιχνεύθηκε, όχι ότι το ανιγόνο δεν υπήρχε στα κύτταρα/στον ιστό που εξετάστηκε. Εάν είναι απαραίτητο, χρησιμοποιήστε μια σειρά αντισωμάτων για την αναγνώριση ψευδώς αρνητικών αντιδράσεων.

## Αναμενόμενα Αποτελέσματα

### Φυσιολογικοί Ιστοί

Ο κλώνος 6F11 ανιχνεύει το ανιγόνο του άλφα υποδοχέα ιστρογόνου (ER) στους πυρήνες των κυττάρων που εμφανίζουν υψηλά επίπεδα έκφρασης ER, μεταξύ των οποίων ένα τμήμα των κυττάρων του ενδομητρίου, των ωοθηκών και του μυομητρίου, καθώς και των πορογενών κυττάρων του φυσιολογικού μαστού. Μπορεί επίσης να παρατηρηθεί χρώση και στη βλεννογόνο της αμυγδαλής, καθώς και σε ένα τμήμα λεμφοειδών κυττάρων βλαστικού κέντρου (Συνολικός αριθμός φυσιολογικών δειγμάτων που αξιολογήθηκαν =136).

### Καρκινικοί Ιστοί

Ο κλώνος 6F11 χρωμάτισε 137/222 όγκους προς αξιολόγηση, στους οποίους περιλαμβάνονταν όγκοι του μαστού (131/180), θηλώδη καρκινώματα του θυρεοειδούς (3/4), όγκοι των ωοθηκών (2/4), μεταξύ των οποίων 1/2 κυσταδενοκαρκινώματα και 1/1 διαινοκυτταρικά καρκινώματα), μεταστατικοί όγκοι αγγύστου προέλευσης (1/2), όγκοι του πνεύμονα (0/4), όγκοι του ήπατος (0/4), όγκοι του εγκέφαλου (0/2), καρκινώματα εκ πλακωδών κυττάρων του οισοφάγου (0/2), αδενοκαρκινώματα του στομάχου (0/2), όγκοι μαλακών μορίων (0/2), καρκινώματα εκ πλακωδών κυττάρων της γλώσσας (0/2), νεφροκυτταρικά καρκινώματα (0/2), καρκινώματα εκ πλακωδών κυττάρων του τραχήλου (0/2), σεμινώματα των όρχεων (0/2), αδενοκαρκινώματα του παχέος εντέρου (0/2), αδενοκαρκινώματα του νεφρού (0/2), όγκοι του δέρματος (0/2), καρκινώματα εκ πλακωδών κυττάρων του λάρυγγα (0/1) και άτυποι καρκινώδεις όγκοι του θύμου αδένος (0/1). (Συνολικός αριθμός όγκων που αξιολογήθηκαν = 222).

**Το NCL-L-ER-6F11 συνιστάται για τον προσδιορισμό της κατάστασης του α-υποδοχέα των ιστρογόνων του καρκινικού ιστού του μαστού.**

## Γενικοί Περιορισμοί

Η ανοσοϊστοχημεία είναι μια διαγνωστική διεργασία πολλαπλών βημάτων, η οποία αποτελείται από ειδικευμένη εκπαίδευση στην επιλογή των κατάλληλων αντιδραστηρίων, επιλογή ιστού, μονιμοποίηση και επεξεργασία, προετοιμασία της πλάκας IHC και ερμηνεία των αποτελεσμάτων της χρώσης.

Η χρώση του ιστού εξαρτάται από το χειρισμό και την επεξεργασία του ιστού πριν από τη χρώση. Τυχόν εσφαλμένη μονιμοποίηση, κατάμειξη, απόψυξη, πλύση, στέγνωμα, θέρμανση, τομή ή μόλυνση με άλλους ιστούς ή υγρά ενδέχεται να παράγει μορφώματα, παγίδευση αντισώματος ή ψευδώς αρνητικά αποτελέσματα. Τυχόν ασυνεπή αποτελέσματα ενδέχεται να οφείλονται σε παραλλαγές των μεθόδων μονιμοποίησης και εγκλεισμού ή σε εγγενείς ανωμαλίες εντός του ιστού.<sup>4</sup>

Τυχόν υπερβολική ή ατελής αντίχρωση ενδέχεται να διακυβεύσει τη σωστή ερμηνεία των αποτελεσμάτων.

Η κλινική ερμηνεία οποιασδήποτε χρώσης ή της απουσίας της θα πρέπει να συμπληρώνεται με μορφολογικές μελέτες που χρησιμοποιούν σωστούς μάρτυρες και θα πρέπει να αξιολογείται στα πλαίσια του κλινικού ιστορικού του ασθενούς και άλλων διαγνωστικών εξετάσεων από ειδικευμένο παθολογοανατόμο.

Τα αντι σώματα που παρέχονται από την Leica Biosystems Newcastle Ltd προορίζονται για χρήση, όπως υποδεικνύεται, είτε σε κατεψυγμένες είτε σε εγκλιμασμένες σε παραφίνη τομές, με ειδικές απαιτήσεις μονιμοποίησης. Ενδέχεται να παρουσιαστεί μη αναμενόμενη εκφάραση αντιγόνου, ειδικά σε νεοπλασμάτα. Η κλινική ερμηνεία οποιασδήποτε χρωματισμένης τομής ιστού πρέπει να περιλαμβάνει μορφολογική ανάλυση και την αξιολόγηση των κατάλληλων μαρτύρων.

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## Τροποποιήσεις Στην Προηγούμενη Έκδοση

Συστάσεις Για Τη Χρήση, Αναμενόμενα Αποτελέσματα.

## Ημερομηνία Έκδοσης

20 Σεπτεμβρίου 2012

# Novocastra™ Væskeformigt Monoklonalt Museantistof

## Estrogen Receptor

### Produktkode: NCL-L-ER-6F11

#### Tilsligtet Anvendelse

*Til in vitro diagnostisk anvendelse.*

NCL-L-ER-6F11 er beregnet til kvalitativ identifikation af Estrogen Receptor-molekyler i paraffinsnit ved lysmikroskopi. Klinisk fortolkning af farvning eller mangel derpå skal suppleres med morfologiske undersøgelser under anvendelse af passende kontroller og bør evalueres i sammenhæng med patientens kliniske historie og andre diagnostiske tests af en kvalificeret patolog.

#### Procedureprincip

Immunhistokemiske (IHC) farvningsteknikker muliggør visualisering af antigener via sekventiel tilsætning af et specifikt antistof mod antigenet (primært antistof), et sekundært antistof mod det primære antistof og et enzym kompleksbundet til et kromogent substrat med indskudte vasketrin. Den enzymatiske aktivering af kromogenet resulterer i et synligt reaktionsprodukt på antigenstedet. Prøven kan derefter kontrastfarves og dækkes med et dækglas. Resultaterne fortolkes ved anvendelse af et lysmikroskop og medvirker til differential diagnose af patofysiologiske processer, som muligvis kan være associeret med et bestemt antigen.

#### Klon

6F11

#### Immunogen

Prokaryotisk rekombinant protein svarende til fuld længde alpha-form af det humane østrogenreceptormolekyle.

#### Specifitet

Human østrogenreceptor.

#### Reagenssammensætning

NCL-L-ER-6F11 er en flydende væskultursupernatant indeholdende natriumazid som konserveringsmiddel.

#### Ig-klasse

IgG1

#### Totalproteinkoncentration Total Protein

Se etiketten på hætteflasken for lotspecifik totalproteinkoncentration.

#### Antistofkoncentration

Større end eller lig med 67,5 mg/l som bestemt ved ELISA. Se etiketten på hætteflasken for lotspecifik Ig-koncentration.

#### Anbefalinger Vedrørende Anvendelse

Immunhistokemi på paraffinsnit.

**Varmeinduceret epitopgenfinding (HIER):** Følg venligst vejledningen i Novocastra Epitope Retrieval Solution pH 6.

**Foreslået fortynding:** 1:50 ved 30 minutter ved 25 °C. Disse retningslinjer er vejledende, og brugeren bør selv bestemme egne optimale brugsopløsninger.

**Visualisering:** Følg venligst vejledningen i Novolink™ Polymer Detection Systems. Yderligere produktinformation og support fås ved henvendelse til lokal forhandler eller Leica Biosystems regionskontor - samt på vores hjemmeside: [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)  
Dette antistofs funktion bør valideres, når det anvendes med andre manuelle farvningssystemer eller automatiserede platforme.

#### Opbevaring Og Holdbarhed

Opbevares ved 2–8 °C. Må ikke fryses. Sættes tilbage til 2–8 °C umiddelbart efter brug. Må ikke anvendes efter udløbsdatoen angivet på hætteflaskens etikette. Andre opbevaringsbetingelser end de ovenfor angivne skal verificeres af brugeren.

#### Prøveklargøring

Det anbefalede fiksativ er 10% neutralbufferjusteret formalin til paraffinindstøbte vævssnit.

#### Advarsler Og Forholdsregler

Dette reagens er fremstillet ud fra supernatanten af en cellekultur. Da det er et biologisk produkt, skal der tages fornuftige sikkerhedsforanstaltninger ved dets håndtering.

Denne reagens indeholder natriumazid. Et datablad for materialesikkerhed kan fås efter anmodning eller er tilgængeligt på [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Konsulter landsdækkende og lokale love og regler vedrørende bortskaffelse af alle potentielt toksiske komponenter.

Prøver skal før og efter fiksering, lige som alle materialer eksponeret mod prøverne, håndteres som potentielt smittefarlige og bortskaffes under iagttagelse af passende forholdsregler<sup>1</sup>. Pipetter aldrig reagenser med munden og undgå, at reagenser og prøver kommer i kontakt med huden eller slimhinder. Hvis reagenser eller prøver kommer i kontakt med følsomme områder, skal der skylles efter med rigelige mængder vand. Søg læge.

Minimer mikrobiel kontaminering af reagenserne, da der ellers kan forekomme øget uspecifik farvning.

Inkubationstider eller -temperaturer andre end de specificerede kan give fejlagtige resultater. Alle sådanne ændringer skal valideres af brugeren.

### Kvalitetskontrol

Forskelle i behandlingen af væv og forskelle i tekniske procedurer i brugerens laboratorium kan frembringe betydeligt varierende resultater og nødvendiggøre regelmæssig udførelse af kontroller på stedet ud over nedenstående procedurer.

Kontrollerne skal være friske autopsier/biopsier/kirurgiske prøver fikseret i formalin og behandlet og indstøbt i paraffin så hurtigt som muligt på samme måde som patientprøver.

### Positiv Vævskontrol

Anvendes til påvisning af, at vævet er fremstillet korrekt, og at der er anvendt korrekte farvningsteknikker.

Der bør inkluderes en positiv vævskontrol for hvert sæt testbetingelser i hver farvekørsel.

Svagt positivt farvet væv er mere egnet end kraftigt positivt farvet væv til optimal kvalitetskontrol og påvisning af små niveauer af reagensnedbrydning.<sup>2</sup>

Anbefalet positivt kontrolvæv er livmoderslimhinde.

Hvis den positive vævskontrol ikke udviser positiv farvning, skal resultater af testprøverne kasseres.

### Negativ Vævskontrol

Skal undersøges efter den positive vævskontrol for at sikre, at det primære antistof mærker målantigenet specifikt.

Det anbefalede negative kontrolvæv er tonsil.

Alternativt frembyder de mange forskellige celletyper, der er til stede i de fleste vævssnit, ofte negative kontrolsteder, men dette skal verificeres af brugeren.

Uspecifik farvning har, hvis til stede, ofte et diffust udseende. Sporadisk farvning af bindevæv kan ligeledes observeres i vævssnit af væv, der er fikseret for kraftigt i formalin. Anvend intakte celler til fortolkning af farvningsresultaterne. Nekrotiske eller degenererede celler farves ofte mere uspecifikt.<sup>3</sup> Der kan eventuelt ses falske positive resultater, der skyldes non-immunologisk binding af proteiner eller substratreaktionsprodukter. Dette kan ligeledes skyldes endogene enzymer, såsom pseudoperoxidase (erythrocytter), endogen peroxidase (cytochrom C) eller endogent biotin (f.eks. lever, bryst, hjerne, nyre) afhængigt af den anvendte type immunfarve. For at differentiere mellem endogen enzymaktivitet eller uspecifik enzymbinding og specifik immunreaktivitet kan yderligere patientvæv eventuelt farves udelukkende med henholdsvis substratkromogen eller enzymkomplekser (avidin-biotin, streptavidin, mærket polymer) og substratkromogen. Hvis der optræder specifik farvning i den negative vævskontrol, skal resultaterne af patientprøverne kasseres.

### Negativ Reagenskontrol

Anvend en uspecifik negativ reagenskontrol i stedet for det primære antistof på et vævssnit af hver patientprøve for at vurdere uspecifik farvning og muliggøre bedre fortolkning af specifik farvning på antigenstedet.

### Patientvæv

Eksaminer patientprøver farvet med NCL-L-ER-6F11 sidst. Intensiteten af positiv farvning bør vurderes i sammenhæng med eventuel uspecifik baggrundsfarvning af den negative reagenskontrol. Som med alle immunhistokemiske tests betyder et negativt resultat, at antigenet ikke blev påvist. Ikke at antigenet var fraværende i de analyserede celler eller det analyserede væv. Om nødvendigt anvendes et panel af antistoffer til identifikation af falske negative reaktioner.

### Forventede Resultater

#### Normalt væv

Klon 6F11 påviser østrogenreceptor (ER) alfa-antigen i cellekernerne, der giver udtryk for høje ER-niveauer, inklusive en andel af endometrielle, ovariale og myometrielle celler samt normale duktalet celler fra brystet. Farvning kan også ses i tonsilslimhinden og en del af kimcenterets lymfoceller. (Samlet antal evaluerede normale tilfælde = 136).

#### Tumørvæv

Klon 6F11 farvede 137/222 evaluerede tumorer, inklusive brysttumorer (131/180), papillære thyroideakarcinomer (3/4), ovariale tumorer (2/4), inklusive 1/2 cystadenokarcinomer og 1/1 klare cellekarcinomer), metastatiske tumorer af ukendt oprindelse (1/2), lungetumorer (0/4), levertumorer (0/4), hjernemetorer (0/2), pladecellekarcinomer i øsofagus (0/2), maveadenokarcinomer (0/2), blodvævstumorer (0/2), pladecellekarcinomer i tungen (0/2), nyre-cellekarcinomer (0/2), pladecellekarcinomer i livmoderhalsen (0/2), testikelseminomer (0/2), tyktarmsadenokarcinomer (0/2), rektale adenokarcinomer (0/2), hudtumorer (0/2), pladecellekarcinomer i larynx (0/1) og atypiske karcinoid tumorer i thymus (0/1). (Samlet antal evaluerede tumortilfælde = 222).

#### NCL-L-ER-6F11 anbefales til bestemmelse af østrogenreceptorens alfa-status i brystcancer-væv.

### Generelle Begrænsninger

Immunhistokemi er en diagnostisk proces bestående af mange trin, der omfatter specialiseret uddannelse i valg af passende reagenser, vævsselektion, -fiksering og -behandling samt fremstilling af IHC-objektglas og fortolkning af farvningsresultaterne.

Vævsfarvning er afhængig af håndteringen og behandlingen af vævet inden farvning. Forkert fiksering, frysning, optøning, vask, tørring, opvarmning, sektionering eller kontaminering med andet væv eller andre væsker kan frembringe artefakter, indfangning af antistof eller falske negative resultater. Inkonsistente resultater kan skyldes variationer i fikserings- og indstøbningsmetoder eller irregulariteter indeholdt i vævet.<sup>4</sup>

For kraftig eller ukomplet kontrastfarvning kan gøre korrekt fortolkning af resultaterne vanskelig.

Klinisk fortolkning af farvning eller mangel derpå skal suppleres med morfologiske undersøgelser under anvendelse af passende kontroller og bør evalueres i sammenhæng med patientens kliniske historie og andre diagnostiske tests af en kvalificeret patolog.

Antistoffer fra Leica Biosystems Newcastle Ltd er som angivet beregnet til anvendelse på enten frosne eller paraffinindstøbte vævssnit med specifikke krav til fiksering. Der kan forekomme uventet antigenekspression, navnlig i neoplasmer. Den kliniske fortolkning af alle farvede vævssnit skal indfatte morfologisk analyse og evaluering af passende kontroller.

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## Rettelser Til Tidligere Udgave

Anbefalingen Vedrørende Anvendelse, Forventede Resultater.

## Udgivelsesdato

20 september 2012

# Novocastra™ Vloeistof Muis Monoklonaal Antilichaam Estrogen Receptor

Productcode: NCL-L-ER-6F11

## Beoogd Gebruik

Voor gebruik bij *in-vitro*-diagnostiek.

NCL-L-ER-6F11 is bedoeld voor de kwalitatieve identificatie door optische microscopie van Oestrogeen Receptor moleculen in paraffinecoupes. De klinische interpretatie van iedere kleuring of de afwezigheid ervan moet worden aangevuld met morfologisch onderzoek en goede controles. De interpretatie moet worden geëvalueerd door een vakkundige patholoog binnen de context van de klinische geschiedenis van de patiënt en eventueel ander diagnostisch onderzoek.

## Beingsel van de Procedure

Immunohistochemische (IHC) kleuringstechnieken maken de visualisatie van antigenen mogelijk via de sequentiële toepassing van een specifiek antilichaam naar het antigeen (primaire antilichaam), het secundaire antilichaam naar het primaire antilichaam en een enzymcomplex met een chromogeen substraat met ingevoegde wasstappen. De enzymatische activering van de chromogeenresultaten in een zichtbaar reactieproduct op de antigene plaats. De monsters kunnen dan tegengekleurd en afgedekt zijn. De resultaten worden geïnterpreteerd met een lichtmicroscop en hulpmiddelen in de differentiële diagnose van pathofysiologische processen, die wel of niet met een specifiek antigeen geassocieerd kunnen worden.

## Kloon

6F11

## Immunogeen

Prokaryotische recombinante eiwit overeenkomt met de volledige lengte alfa-vorm van de menselijke oestrogeen receptor molecuul.

## Specificiteit

Menselijke oestrogeen receptor.

## Reagentiasamenstelling

NCL-L-ER-6F11 is een supernatant van de vloeibare weefselweek die natriumazide bevat als conserveringsmiddel.

## Ig-klasse

IgG1

## Totale Proteïneconcentratie

Total Protein

Raadpleeg het etiket op de flacon voor de specifieke totale proteïneconcentratie.

## Antilichaamconcentratie

Groter of gelijk aan 67.5 mg/L zoals bepaald door ELISA. Raadpleeg het etiket op de flacon voor de specifieke Ig-concentratie.

## Aanbevelingen over het Gebruik

Immunochemisch op paraffine coupes.

**Warme-geïnduceerd epitoopherstel (HIER):** Volg de instructies voor gebruik in Novocastra Epitope Retrieval Solution pH 6.

**Aangeraden verdunning:** 1:50 voor 30 minuten bij 25 °C. Dit wordt gezien als een richtlijn en gebruikers dienen hun eigen optimale werkverdunningen te bepalen.

**Visualisatie:** Volg a.u.b. de gebruiksinstructies in de Novolink™ Polymer Detection Systems. Voor meer productinformatie of ondersteuning dient u contact op te nemen uw lokale distributeur of het regionale kantoor van Leica Biosystems, of de website van Leica Biosystems te bezoeken, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

De prestatie van dit antilichaam dient gevalideerd te worden als het wordt gebruikt met andere handmatige kleuringssystemen of automatische platformen.

## Opslag en Stabiliteit

Opslaan bij temperaturen van 2–8 °C. Niet bevriezen. Laat het systeem direct na gebruik terugkeren naar een temperatuur van 2–8 °C. Gebruik het product niet meer na de expiratedatum die op de flacon staat. Opslagcondities andere dan degene die hierboven gespecificeerd zijn, dienen door de gebruiker geverifieerd te.

## Vorbereiding van Monsters

De aanbevolen fixeerstof is 10% neutraal gebufferde formaline voor paraffine ingebedde weefselcoupes.

## Waarschuwingen en Voorzorgsmaatregelen

Deze reagens is voorbereid van het supernatant van de celweek. Aangezien het biologisch product is, dient u bij het gebruik ervan voorzichtig te werk te gaan.

Deze reagens bevat natriumazide. Een materiaalveiligheidsblad is op verzoek verkrijgbaar bij [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Raadpleeg de richtlijnen van de lokale of nationale overheid voor het afhandelen van potentieel giftige componenten.

Monsters moeten voor en na fixatie worden behandeld als potentiële overdragers van infecties en volgens de juiste voorzorgsmaatregelen worden afgedankt. Dit geldt tevens voor alle materialen die aan de monsters zijn blootgesteld.<sup>1</sup>

Reagentia mogen nooit met de mond worden gepipetteerd. Daarnaast moet contact tussen de huid en het slijmvlies met reagentia en monsters worden vermeden.

Als reagentia of monsters in contact komen met gevoelige gebieden, moet u deze gebieden wassen met een ruime hoeveelheid water. Neem contact op met een arts.

Minimaliseer de kans van microbacteriële contaminatie van reagentia. Als u dit niet doet, kan er een toename van niet-specifieke kleuring optreden.

Incubatietijden of temperaturen die afwijken van degenen die gespecificeerd zijn, kunnen tot onjuiste resultaten leiden. Iedere dergelijke verandering moet door de gebruiker gevalideerd worden.

### **Kwaliteitscontrole**

Verschillen in het verwerken van weefsel en technische procedures in het laboratorium van de gebruiker kunnen zorgen voor een aanzienlijke variabiliteit van de resultaten. Dit vereist een regulier gebruik van bedrijfseigen controles naast de volgende procedures.

De controles moeten verse autopsie-, biopsie-, of chirurgische monsters omvatten, en zo snel mogelijk formaline gefixeerd en in paraffinewax ingebed worden, op dezelfde manier als de patiëntmonster(s).

### **Positieve Weefselcontrole**

Wordt gebruikt om correct voorbereide weefsels en goede kleuringstechnieken aan te duiden.

Er dient een positieve weefselcontrole opgenomen te worden voor iedere set testcondities in iedere kleuringsrun.

Voor een optimale kwaliteitscontrole en voor het detecteren van geringe niveaus van reagensdegradatie, is weefsel met zwakke positieve kleuring beter geschikt dan weefsel met sterke positieve kleuring.<sup>2</sup>

Aanbevolen positieve weefselcontrole is baarmoederslijmvlies.

Als de positieve weefselcontrole geen positieve kleuring vertoont, moeten de resultaten met de testmonsters als ongeldig worden beschouwd.

### **Negatieve Weefselcontrole**

Dient onderzocht te worden na de positieve weefselcontrole om de specificiteit te verifiëren van de labeling van het doelantigen door het primaire antilichaam.

Aanbevolen negatieve weefselcontrole is tonsillen.

Daarnaast leveren de verscheidenheid aan celtypen, die in de meeste weefselcoupes aanwezig zijn, regelmatig negatieve controlelocaties op, maar dit dient door de gebruiker geverifieerd te worden. Niet-specifieke kleuring, indien aanwezig, heeft meestal een diffuus uiterlijk.

Daarnaast kan in coupes sporadische kleuring van bindweefsel worden geobserveerd. Dit treedt op als gevolg van overdadig fixeren van weefsel met formaline. Maak voor de interpretatie van kleuringsresultaten gebruik van intacte cellen. Necrotische of gedegenereerde cellen kunnen vaak een niet-specifieke kleuring vertonen.<sup>3</sup>

Er kan sprake zijn van fout-positieven als gevolg van niet-immunologische binding van eiwitten of substraatreactieproducten. Zij kunnen ook veroorzaakt worden door endogene enzymen zoals pseudoperoxidase (erythrocyten), endogene peroxidase (cytochrom C), of endogene biotine (bijv. lever, borst, hersenen, nieren), afhankelijk van het type immunokleuring dat gebruikt wordt.

Om endogene enzymen of niet-specifieke binding van enzymen van specifieke immunoreactiviteit te differentiëren, kan het zijn dat extra patiëntweefsels exclusief gekleurd wordt met substraat chromogeen of enzymcomplexen (avidine-biotine, streptavidine, gelabeld polymeer) en respectievelijk substraat-chromogeen. Indien specifieke kleuring binnen het interne negatieve controleweefsel optreedt, moeten de resultaten die met de patiëntmonsters zijn verkregen als ongeldig worden beschouwd.

### **Negatieve Reagenscontrole**

Gebruik een niet-specifieke negatieve reagenscontrole in plaats van het primaire antilichaam met een coupe van ieder patiëntmonster, om een niet-specifieke kleuring te evalueren en een betere interpretatie te krijgen van de specifieke kleuring op de antigene plaats.

### **Patiëntweefsel**

Onderzoek de gekleurde patiëntmonsters met NCL-L-ER-6F11. De positieve kleuringsintensiteit moet worden geëvalueerd binnen de context van iedere niet-specifieke achtergrondkleuring van de negatieve reagenscontrole. Net zoals bij elke immunohistochemische test betekent een negatief resultaat dat het antigeen niet is gedetecteerd. Het betekent dus niet dat het antigeen afwezig was in de geanalyseerde cellen/het geanalyseerde weefsel. Gebruik een panel van antilichamen om de verkeerd-negatieve reacties te identificeren.

### **Verwachte Resultaten**

#### Normale weefsels

Kloon 6F11 detecteert de oestrogeenreceptor (ER) alfa-antigeen in de celkernen die een hoge mate van ER vertonen, naast een proportie endometriale, ovariale en myometriale cellen en normale ductale borstcellen. Kleuring kan ook worden waargenomen in de amandelslijmvliezen en een deel van de lymfoïde cellen van de kliercentra. (Totaal aantal geëvalueerde normale gevallen = 136).

#### Abnormale weefsels

Kloon 6F11 kleurde 137/222 onderzochte tumoren, waaronder borsttumoren (131/180), papillaire schildklier carcinoomen (3/4), ovariale tumoren (2/4, waarvan 1/2 cystadenocarcinomen en 1/1 'clear cell'-carcinomen), metastatische tumoren van onbekende oorsprong (1/2), longtumoren (0/4), levertumoren (0/4), hersentumoren (0/2), squameuze celcarcinomen van de slokdarm (0/2), adenocarcinomen van de maag (0/2), tumoren van de weke delen (0/2), squameuze celcarcinomen van de tong (0/2), niercelcarcinomen (0/2), squameuze celcarcinomen van de baarmoederhals (0/2), testiculaire seminomen (0/2), adenocarcinomen van de dikke darm (0/2), rectale adenocarcinomen (0/2), huidtumoren (0/2), squameuze celcarcinomen van het strottenhoofd (0/1) en atypische carcinoïde tumoren van de thymus (0/1). (Totaal aantal geëvalueerde tumorgevallen = 222).

**NCL-L-ER-6F11 wordt aanbevolen voor het bepalen van de oestrogeen receptor alfa-status van borstkanker weefsels.**

## Algemene Beperkingen

Immunohistochemie is een diagnoseproces van meerdere stappen dat uit een gespecialiseerde training bestaat in het selecteren van de desbetreffende reagentia; weefselselectie, fixatie en verwerking; voorbereiding van de IHC-objectglasjes; en de interpretatie van de kleuringresultaten. Weefselkleuring is afhankelijk van het gebruik en de verwerking van het weefsel vóór het aanbrengen van de kleuring. Een onjuiste manier van fixeren, invriezen, ontdoeien, wassen, drogen, verwarmen en opdelen of contaminatie met andere weefsels of vloeistoffen kunnen leiden tot artefacten, het vastzitten van antilichamen of fout-negatieven. Inconsistente resultaten kunnen het gevolg zijn van variaties in de methoden die voor het fixeren en inbedden worden gebruikt of van inherente onregelmatigheden binnen het weefsel.<sup>4</sup>

Overmatige of onvolledige tegenkleuring kan een correcte interpretatie van de resultaten in te weg zitten.

De klinische interpretatie van iedere kleuring of de afwezigheid ervan moet worden aangevuld met morfologisch onderzoek en goede controles. De interpretatie moet worden geëvalueerd door een vakkundige patholoog binnen de context van de klinische geschiedenis van de patiënt en eventueel ander diagnostisch onderzoek.

Antilichamen van Leica Biosystems Newcastle Ltd zijn bedoeld voor gebruik, zoals aangegeven, op bevoren of paraffine ingebedde coupes met specifieke fixatie-eisen. Er kan een onverwachte antigenexpressie optreden, met name in neoplasma's. De klinische interpretatie van ieder gekleurde weefselcoupe moet morfologische analyses bevatten en de evaluatie van de juiste controles.

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## Aanpassingen ten opzichte van Vorige Editie

Aanbevelingen over het Gebruik, Verwachte Resultaten.

## Publicatiedatum

20 september 2012



# Novocastra™ Flytende Monoklonalt Antistoff Fra Mus Estrogen Receptor

## Produktkode: NCL-L-ER-6F11

### Tiltenkt bruk

*Til in vitro-diagnostisk bruk.*

NCL-L-ER-6F11 skal brukes til kvalitativ identifikasjon av Østrogen reseptor molekyler i parafinsnitt ved lysmikroskopi. Den kliniske tolkningen av farge eller manglende farge skal suppleres med morfologiske undersøkelser og bruk av egnede kontroller, og bør evalueres av en kvalifisert patolog i lys av pasientens kliniske historie og eventuelle andre diagnostiske tester.

### Prosedyreprinsipp

Immunhistokjemiske (IHC) fargingsteknikker gjør det mulig å se antigener via en sekvensiell tilsetning av et bestemt antistoff mot antigenet (primært antistoff), et sekundært antistoff mot det primære antistoffet og et enzymkompleks med et kromogent substrat med innskutte vasketrinn. Den enzymatiske aktiveringen av kromogenet gir et synlig reaksjonsprodukt på antigenstedet. Prøven kan deretter kontrastfarges og dekkes med et dekkglass. Resultatene fortolkes ved hjelp av et lysmikroskop og medvirker til differensialdiagnose av patofysiologiske prosesser som muligens kan være assosiert med et bestemt antigen.

### Klon

6F11

### Immunogen

Prokaryot rekombinant proteinet som tilsvarer full lengde alfa form av det menneskelige østrogen reseptor molekyll.

### Spesifisitet

Menneskelig østrogen reseptor.

### Reagenssammensetning

NCL-L-ER-6F11 er en flytende vevskultursupernatant som inneholder natriumazid som konserveringsmiddel.

### Ig-klasse

IgG1

### Totalproteinkonsentrasjon

Total Protein

Se etiketten på hetteglasset for lotspesifikk totalproteinkonsentrasjon.

### Antistoffkonsentrasjon

Større enn eller tilsvarende 67.5 mg/l i henhold til ELISA. Se etiketten på hetteglasset for lotspesifikk Ig-konsentrasjon.

### Anbefalinger for Bruk

Immunhistokjemi på parafinsnitt.

**Varmeindusert epitopgjengvinning (HIER):** Følg instruksjonene for bruk i Novocastra Epitope Retrieval Solution pH 6.

**Foreslått fortykning:** 1:50 i 30 minutter ved 25 °C. Disse retningslinjene er veiledende, og brukeren bør selv bestemme egne optimale bruksfortynninger.

**Visualisering:** Følg bruksanvisningen for Novolink™ Polymer Detection Systems. Ønsker du ytterligere produktinformasjon eller -støtte, kan du ta kontakt med den lokale forhandleren eller regionkontoret til Leica Biosystems, eller på nettsidene til Leica Biosystems, [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Ytelsen til dette antistoffet bør valideres ved bruk av andre manuelle fargingsystemer eller automatiske systemer.

### Oppbevaring og Stabilitet

Oppbevares ved 2–8 °C. Må ikke fryses. Returneres til 2–8 °C umiddelbart etter bruk. Må ikke brukes etter utløpsdatoen angitt på produktetiketten. Andre oppbevaringsbetingelser må valideres av brukeren.

### Klargjøring av Prøver

Anbefalt fiksativ er 10 % nøytralbufret formalin for parafinlagrede vevsnett.

### Advarsler og Forholdsregler

Denne reagensen er laget av supernatanten fra en cellekultur. Dette er et biologisk produkt som må behandles deretter.

Denne reagensen inneholder natriumazid. Dataark om materialsikkerhet (MSDS) er tilgjengelig på forespørsel eller kan lastes ned fra [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com)

Følg nasjonale og lokale forskrifter for avhending av komponenter som kan være giftige.

Prøver (før og etter fiksering) og alt materiale som eksponeres for dem, skal behandles som potensielt smittefarlig og kasseres i samsvar med gjeldende forholdsregler.<sup>1</sup>

Hold aldri pipetter med reagens i munnen, og unngå at hud og slimhinner kommer i kontakt med reagenser og prøver.

Hvis reagenser eller prøver kommer i kontakt med følsomme områder, skal de skylles med rikelig vann. Kontakt lege.

Reduser mikrobiell kontaminering av reagensene til et minimum, ellers kan det forekomme økt uspesifisert farging.

Inkubasjonstid eller temperaturer som er annerledes enn det som er angitt, kan gi unøyaktige resultater. Slike endringer må valideres av brukeren.

## Kvalitetskontroll

Forskjeller i behandlingen av vev og forskjeller i tekniske prosedyrer i brukerens laboratorium kan gi signifikant varierte resultater, og det kan være nødvendig å foreta kontroller på stedet i tillegg til prosedyrene angitt nedenfor.

Kontrollene skal være nye autopsi-/biopsi-/kirurgiske prøver, formalinfixerte, behandlede og parafinlagrede så snart som mulig, på samme måte som pasientprøver.

## Positiv Vevskontroll

Brukes for å påvise korrekt vevspreparering og fargeteknikker.

En positiv vevskontroll bør inkluderes for hvert sett med testbetingelser i hver fargerunde.

Svakt positivt farget vev er mer egnet enn kraftig positivt farget vev til optimal kvalitetskontroll og påvisning av små nivåer reagensnedbrytning.<sup>2</sup>

Anbefalt positivt kontrollvev er livmorslimhinnen.

Hvis den positive vevskontrollen ikke viser positiv farging, skal resultatene til testprøvene anses som ugyldige.

## Negativ Vevskontroll

Skal undersøkes etter den positive vevskontrollen for å sikre at det primære antistoffet merker målantigenet spesifikt.

Anbefalt negativt kontrollvev er mandel.

Alternativt har de mange ulike celletypene som finnes i de fleste vevssnittene ofte negative kontrollsteder, men dette må verifiseres av brukeren. Uspesifikk farging, hvis dette er aktuelt, har ofte et diffus utseende.

Sporadisk farging av bindevev kan på samme måte observeres i snitt fra vev som er fixert for kraftig i formalin. Bruk intakte celler for å tolke fargerresultatene. Nekrotiske eller degenererte celler kan ofte farges uspesifikt.<sup>3</sup>

Falske positive resultater kan skyldes ikke-immunologisk binding av proteiner eller substratreaksjonsprodukter. Dette kan også skyldes endogene enzymer som pseudoperoksidase (erythrocytter), endogen peroksidase (cytokrom C) eller endogent biotin (f.eks. lever, bryst, hjerne, nyre), avhengig av anvendt type immunfarge.

For å differensiere endogen enzymaktivitet eller uspesifikk enzymbinding og spesifikk immunreaktivitet kan ytterligere pasientvev eventuelt farges kun med henholdsvis substratkromogen eller enzymkomplekser (avidin-biotin, streptavidin, merket polymer) og substratkromogen. Hvis det skjer spesifikk farging i den negative vevskontrollen, må resultatene for pasientprøvene anses som ugyldige.

## Negativ reagenskontroll

Bruk en uspesifikk negativ reagenskontroll i stedet for det primære antistoffet på et snitt av hver pasientprøve for å vurdere uspesifikk farging og for å muliggjøre bedre fortolkning av spesifikk farging på antigenstedet.

## Pasientvev

Undersøk pasientprøver farget med NCL-L-ER-6F11 sist. Intensiteten av positiv farging bør vurderes i sammenheng med eventuell uspesifikk bakgrunnsfarging av den negative reagenskontrollen. Som med alle immunhistokjemiske tester, betyr et negativt resultat at antigenet ikke ble påvist, ikke at antigenet var fraværende i de analyserte cellene/vevet. Om nødvendig kan man bruke et panel av antistoffer for å identifisere falske negative reaksjoner.

## Forventede Resultater

### Normalt Vev

Klone 6F11 påviser østrogenreseptor (ER) alfaantigen i cellenuclei som uttrykker høye ER-nivåer, inkludert en andel endometriske, ovariale og myometriske celler, samt normale ductceller i bryst. Farging kan også ses i slimhinnen i tonsillene og en andel germinalsenter lymfoide celler. (Totalt antall vurderte, normale tilfeller = 136).

### Abnormalt Vev

Klone 6F11 farget 137 av 222 tumorer som ble vurdert, inkludert brysttumorer (131/180), papillærkarcinomer i skjoldbruskkjertelen (3/4), ovariale tumorer (2/4, inkludert 1/2 cystadenokarcinomer og 1/1 klarcellekarcinomer), metastatiske tumorer av ukjent opprinnelse (1/2) lungtumorer (0/4), levertumorer (0/4), hjernetumorer (0/2), plaeepitelkarcinomer fra spiserør (0/2), adenokarcinomer fra mage (0/2), bløtvevstumorer (0/2), plateepitelcellekarcinomer fra tunge (0/2) nyrecellekarcinomer (0/2), plateepitelcellekarcinom fra livmorhals (0/2), testikkelsemionomer (0/2), adenokarcinomer fra kolon (0/2), adenokarcinomer fra rektum (0/2), hudtumorer (0/2), plateepitelkacinomer fra strupehode (0/1) og atypiske karsinoide tumorer fra thymus (0/1). (Totalt antall vurderte tumortilfeller = 222).

### NCL-L-ER-6F11 anbefales for å bestemme østrogen reseptor alfa status av brystkreft vev.

## Generelle Begrensninger

Immunhistokjemi er en diagnostisk prosess i flere trinn som omfatter spesialutdanning i valg av egnede reagenser, vevsseleksjon, -fiksering og -behandling samt preparering av IHC-objektglass og tolking av fargerresultater. Vevsfarging avhenger av håndteringen og behandlingen av vevet før fargingen. Feil fiksering, frysing, tining, vasking, tørking, oppvarming, snitting eller kontaminering med annet vev eller væsker kan gi artefakter, innfangning av antistoffer eller falske negative resultater. Inkonsekvente resultater kan skyldes variasjoner ved fiksering eller innstøpningsmetoder eller iboende uregelmessigheter i vevet.<sup>4</sup>

Overdreven eller ufullstendig motfarging kan også gjøre det vanskelig å tolke resultatene riktig.

Den kliniske tolkningen av farge eller manglende farge skal suppleres med morfologiske undersøkelser og bruk av egnede kontroller, og bør evalueres av en kvalifisert patolog i lys av pasientens kliniske historie og eventuelle andre diagnostiske tester.

Antistoffer fra Leica Biosystems Newcastle Ltd skal brukes, som angitt, på enten frosne eller parafinlagrede snitt med spesifikke krav til fiksering. Uventet antigenekspresjon kan forekomme, spesielt i neoplasma. Den kliniske tolkningen av fargede vevssnitt må omfatte morfologiske analyser og evaluering av egnede kontroller.

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## Endringer i forhold til Forrige Utgave

Anbefalinger for Bruk, Forventede Resultater.

## Utgivelsesdato

20 september 2012

# Novocastra™ Likit Monoklonal FareAntikor

## Estrogen Receptor

### Ürün Kodu: NCL-L-ER-6F11

#### Kullanım Amacı

*In vitro* diagnostik kullanımı için.

NCL-L-ER-6F11, parafin seksiyonlarında Östrojen reseptörü molekülleri ışık mikroskopisi tarafından kalitatif tanımlama için kullanılmak üzere tasarlanmıştır. Herhangi bir boyamanın mevcut olması veya olmaması ile ilgili klinik yorumlama, uygun kontroller kullanılarak morfolojik çalışmalarla tamamlanmalıdır ve hastanın klinik geçmişi ve diğer diagnostik testler kapsamında kalifiye bir patolojist tarafından değerlendirilmelidir.

#### Prosedür Prensipleri

İmmünohistokimyasal (IHC) boyama teknikleri, spesifik bir antikorun antijene (primer antikor), ikincil bir antikorun primer antikora ve bir enzim kompleksinin kromojenik bir substrat ile arada yıkama adımları olacak şekilde sekansiyel olarak uygulanmasıyla antijenlerin görselleştirilmesini sağlar. Kromojenin enzimatik aktivasyonu, antijen bölgesinde görünür bir reaksiyon ürettiği ile sonuçlanır. Numune bu durumda karşıt boyanabilir ve lamellenebilir. Sonuçlar, bir ışık mikroskopu kullanılarak yorumlanır ve özel bir antijenle birleştirilebilen veya birleştirilemeyen patofizyolojik işlemlerin ayrıştırıcı tanısına yardımcı olur.

#### Clone

6F11

#### İmmünojen

İnsan östrojen reseptör molekülünün tam uzunlukta alfa formuna karşılık gelen prokaryotik rekombinant proteini.

#### Spesifite

İnsan östrojen reseptörü.

#### Reagent Kompozisyonu

NCL-L-ER-6F11, prezervatif olarak sodyum azit içeren supernatant bir likit doku kültürüdür.

#### Ig Sınıfı

IgG1

#### Toplam Protein Konsantrasyonu

Total Protein

Lota özel toplam protein konsantrasyonu için viyal etiketine başvurun.

#### Antikor Konsantrasyonu

ELISA tarafından belirlendiği gibi 67.5 mg/L'ye eşit veya bu değerdan yüksek. Lota özel Ig konsantrasyonu için viyal etiketine başvurun.

#### Kullanım Tavsiyeleri

Parafin seksiyonlarında immünohistokimya.

**Isı Kaynaklı Epitop Geri Kazanımı (HIER):** Lütfen, Novocastra Epitope Retrieval Solution pH 6 içerisindeki kullanım talimatlarını takip edin.

**Önerilen dilüsyon:** 1:50 25 °C'de 30 dakika için. Bu bir kılavuz olarak verilmiştir; kullanıcılar, kendilerine özel optimal çalışma dilüsyonlarını belirlemelidirler.

**Görselleştirme:** Novolink™ Polymer Detection System kullanım talimatlarına uyun. Ürünle ilgili daha fazla bilgi veya destek için yerel distribütörünüze veya bölgesel Leica Biosystems ofisine başvurun veya alternatif olarak [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com) Leica Biosystems internet sitesini ziyaret edin.

Bu antikorun performansı, diğer manuel boyama sistemleri veya otomatik platformlarla kullanıldığında doğrulanmalıdır.

#### Saklama ve Dayanıklılık

2-8 °C'de saklayın. Dondurmayın. Kullanımdan hemen sonra 2-8 °C'ye dönün. Viyal etiketinin üzerinde belirtilen son kullanım tarihinden sonra kullanmayın. Yukarıda belirtilenlerin dışındaki saklama koşullarının, kullanıcı tarafından kontrol edilmesi gerekir.

#### Numune Hazırlığı

Önerilen fiksatif, parafine gömülmüş doku seksiyonları için %10 nötr tamponlu formalindir.

#### Uyarılar ve Önlemler

Bu reagent, hücre kültürünün supernatantından hazırlanmıştır. Bu bir biyolojik ürün olduğundan işlem yaparken özel dikkat gerektirir.

Bu reagent, sodyum azit içerir. Talep üzerine veya [www.LeicaBiosystems.com](http://www.LeicaBiosystems.com) 'dan bir Material Safety Data Sheet (Malzeme Güvenlik Veri Sayfası) elde edilebilir.

Potansiyel tüm toksik bileşenlerin imhası için federal, ulusal veya lokal düzenlemelere başvurun.

Fikse etme işleminden önce ve sonra numuneler ve bunlara maruz kalan tüm materyaller, enfeksiyon yayabilecek gibi ele alınmalı ve doğru önlemler alınarak atığa çıkartılmalıdır.<sup>1</sup>

Reagent'lar asla ağızla pipetlenmemeli ve cildin ve muköz membranların reagent ve numunelerle temasından kaçınılmalıdır.

Reagent veya numunelerin hassas alanlarla temas etmesi durumunda bu alanları bol su ile yıkayın. Doktora başvurun.

Reagent'ların mikrobiyal kontaminasyonunu minimize edin, aksi durumda nonspesifik boyamada bir artış ortaya çıkabilir.

Belirtilenlerin dışında inkübasyon süreleri veya sıcaklıkları, hatalı sonuçlara neden olabilir. Tüm değişiklikler, kullanıcı tarafından doğrulanmalıdır.

### **Kalite Kontrol**

Kullanıcının laboratuvarındaki doku işleme ve teknik prosedürlerdeki değişiklikler, sonuçlarda önemli farklılıklara neden olabilir ve aşağıdaki prosedürlere ek olarak dahili kontrollerin düzenli şekilde yapılmasını gerektirir.

Kontroller, mümkün olan en kısa sürede ve hasta örneği (örnekleri) ile aynı şekilde formalinle fikse edilmiş, işlenmiş ve parafin mumuna gömülmüş taze topso/biyopsi/cerrahi numune olmalıdır.

### **Pozitif Doku Kontrolü**

Doğru hazırlanmış dokuları ve düzgün boyama tekniklerini belirtmek için kullanılır.

Bir pozitif doku kontrolü, her boyama çalıştırmasında test koşullarının her seti için dahil edilmelidir.

Optimal kalite kontrol için ve reagent degradasyonunun minör düzeylerini tespit etmek için zayıf pozitif boyamaya sahip bir doku, güçlü pozitif boyamaya sahip bir dokudan daha uygundur.<sup>2</sup>

Önerilen pozitif kontrol dokusu: endometriumu.

Pozitif doku kontrolü, pozitif boyamayı göstermezse test numuneleri ile elde edilen sonuçlar geçersiz olarak ele alınmalıdır.

### **Negatif Doku Kontrolü**

Pozitif doku kontrolünden sonra hedef antijenin etiketleme spesifitesini primer antikorla kontrol etmek için gerçekleştirilmelidir.

Önerilen negatif kontrol dokusu: bademcik.

Pek çok doku seksiyonunda bulunan farklı hücre tiplerinin çeşitliliği, genelde negatif kontrol bölgeleri sağlar ancak bu, kullanıcı tarafından kontrol edilmelidir. Nonspesifik boyama, mevcutsa genelde difüz bir görünüme sahiptir.

Bağ dokusu sporadik boyama, aşırı formalinle fikse edilmiş dokulardan seksiyonlarda da gözlemlenebilir. Boyama sonuçlarının yorumlanması için intakt hücreler kullanın. Nekrotik veya dejenerer hücreler, genelde belirsiz şekilde boyanabilir.<sup>3</sup>

Yanlış pozitif sonuçlar, substrat reaksiyon ürünleri veya proteinlerin immünojenik olmayan protein bağlanması nedeniyle görülebilir.

Bunlar, kullanılan immüno boyamanın tipine bağlı olarak psödoperoksidad (eritrositler), endojen peroksidad (sitokrom C) veya endojen biotin (örn. karaciğer, meme, beyin, böbrek) gibi endojen enzimler nedeniyle de ortaya çıkabilir.

Endojen enzim aktivitesini veya enzimlerin nonspesifik bağlanmasını, spesifik immünoreaktiveden ayırt etmek için ilave hasta dokuları, sadece sırasıyla substrat kromojen veya enzim kompleksleriyle (avidin biotin, streptavidin, etiketli polimer) ve substrat kromojen ile boyanabilir. Spesifik boyamann, negatif doku kontrolünde ortaya çıkması durumunda hasta numuneleri ile elde edilen sonuçlar geçersiz olarak ele alınmalıdır.

### **Negatif Reagent Kontrolü**

Antijen bölgede nonspesifik boyamanın değerlendirilmesi ve spesifik boyamanın daha iyi yorumlanmasını sağlamak amacıyla her hasta numunesinin bir seksiyonu ile primer antikorun yerine bir nonspesifik negatif reagent kontrolü kullanın.

### **Hasta Dokusu**

NCL-L-ER-6F11 ile boyanan son hasta numunelerini inceleyin. Pozitif boyama intensitesi, negatif reagent kontrolünün herhangi bir nonspesifik arka plan boyamasının kapsamında değerlendirilmelidir. Herhangi bir immünohistokimyasal test ile negatif bir sonuç, antijenin tespit edilmediği anlamına gelir; antijenin test edilen hücrelerde/dokuda mevcut olmadığı anlamına gelmez. Gerekliyse yanlış negatif reaksiyonları belirlemek için bir antikor paneli kullanın.

### **Öngörülen Sonuçlar**

#### Normal Dokular

6F11 klonu, endometriyal, ovaryen ve miyometriyal hücreleri ile normal göğüs kanalsı hücrelerinin bir oranı da dahil olmak üzere yüksek seviyeleri ifade eden hücre çekirdeklerinde östrojen reseptörü (ER) alfa antijeni tespit etmiştir. Boyama, ayrıca bademcik mukozasında ve germinal merkezi lenfoid hücrelerinin bir kısmında da görülebilir. (Değerlendirilen toplam normal vaka sayısı = 136).

#### Abnormal Dokular

6F11 klonu, göğüs tümörleri (131/180), tiroid papiller karsinomları (3/4), ovaryen tümörleri (2/4, kistadenokarsinomları ve 1/1 şeffaf hücre karsinomları dahil), bilinmeyen kaynaklı metastatik tümörler (1/2), akciğer tümörleri (0/4), karaciğer tümörleri (0/4), beyin tümörleri (0/2), özofagus sküamöz hücre karsinomları (0/2), mide adenokarsinomları (0/2), yumuşak doku tümörleri (0/2), dil sküamöz hücre karsinomları (0/2), renal hücre karsinomları (0/2), serviks sküamöz hücre karsinomları (0/2), testis seminomları (0/2), kolon adenokarsinomları (0/2), rektal adenokarsinomları (0/2), cilt tümörleri (0/2), gırtlak sküamöz hücre karsinomları (0/1) ve timüs tipik olmayan karsinoid tümörleri (0/1) de dahil olmak üzere değerlendirilen tümörlerde 137/222 oranında boyama yapmıştır. (Değerlendirilen toplam tümör vakası sayısı = 222).

**NCL-L-ER-6F11 meme kanseri dokuların östrojen reseptörü alfa statüsünün belirlenmesi için tavsiye edilir.**

### **Genel Sınırlamalar**

İmmünohistokimya uygun reagent'ların seçilmesinde; dokunun seçilmesi, fikse edilmesi ve işlenmesinde; IHC laminin hazırlanmasında ve boyama sonuçlarının yorumlanmasında uzmanlık eğitimi gerektiren çok adımlı bir diagnostik işlemdir. Doku boyama, boyamadan önce dokunun ele alınması ve işlenmesine bağlıdır. Diğer dokularla veya akışkanlarla hatalı fikse etme, dondurma, eritme, yıkama, kurutma, ısıtma, seksiyonlama veya kontaminasyon artefakt, antikor trapping veya yanlış negatif sonuçlar oluşturabilir. Doku içerisinde fikse etme ve gömme yöntemleri veya inherent aksaklıklar nedeniyle tutarsız sonuçlar ortaya çıkabilir.<sup>4</sup>

Aşırı veya inkomplet karşıt boyu, sonuçların doğru yorumlanmasına engel olabilir.

Herhangi bir boyamanın mevcut olması veya olmaması ile ilgili klinik yorumlama, uygun kontroller kullanılarak morfolojik çalışmalarla tamamlanmalıdır ve hastanın klinik geçmişi ve diğer diagnostik testler kapsamında kalifiye bir patolojist tarafından değerlendirilmelidir.

Leica Biosystems Newcastle Ltd antikorları, belirlediği gibi spesifik fikse etme işlemleri gerektiren dondurulmuş veya parafin gömülmüş seksiyonlarda kullanılmak içindir. Özellikle neoplazmalarda beklenmedik antijen ekspresyonu ortaya çıkabilir. Boyanan doku seksiyonunun klinik yorumu, morfolojik analiz ve uygun kontrollerin değerlendirilmesini içermelidir.

## Kaynakça - Genel

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## Önceki Baskıya Göre Değişiklikler

Kullanım Tavsiyeleri, Öngörülen Sonuçlar.

## Yayın tarihi

20 Eylül 2012



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