

Estrogen Receptor Clone 6F11 Ready-To-Use Primary Antibody for Bond™

Catalog No: PA0151 (7 mL) / PA0009 (30 mL)

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Instructions for Use

Please read before using this product.

Check the integrity of the packaging before use.

Estrogen Receptor Clone 6F11

Ready-To-Use Primary Antibody For Bond™

Catalog No: PA0151 (7 mL) / PA0009 (30 mL)

Intended Use

This reagent is for in vitro diagnostic use.

Estrogen Receptor Clone 6F11 (ER 6F11) Mouse Monoclonal antibody is intended for laboratory use to qualitatively identify estrogen receptor (ER) antigen in sections of formalin fixed, paraffin embedded breast cancer tissue by immunohistochemistry methods. Estrogen Receptor Clone 6F11 specifically binds to the ER antigen located in the nucleus of ER positive normal and neoplastic cells.

Estrogen Receptor Clone 6F11 is indicated as an aid in the management, prognosis and predication of therapy outcome of breast cancer. 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.

Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is optimized for use on the Leica Biosystems Bond-III staining platform using the Bond Polymer Refine Detection kit.

Summary and Explanation

ER content of breast cancer tissue is an important parameter in the prediction of prognosis and response to endocrine therapy. The introduction of monoclonal antibodies to ER has allowed the determination of receptor status of breast tumors to be carried out in routine histopathology laboratories. Estrogen Receptor Clone 6F11 is a mouse monoclonal antibody directed against the human estrogen receptor molecule. A prokaryotic recombinant protein, corresponding to the full length human ER molecule was used as the immunogen. Estrogen Receptor Clone 6F11 has been shown to react with a 66 kD protein from MCF-7 cell lysates via Western blot.⁴

Principle of Procedure

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see "Using BOND Reagents" in your BOND user documentation). Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is a ready to use product that has been specifically optimized for use on the automated BOND-III system in combination with Bond Polymer Refine Detection.

The recommended staining protocol for Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is IHC Protocol F. Heat induced epitope retrieval is recommended using Bond Epitope Retrieval Solution 1 for 20 minutes. Bond Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. The detection system avoids the use of streptavidin and biotin, and therefore eliminates non-specific staining as a result of endogenous biotin.

Bond Polymer Refine Detection works as follows:

- The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity
- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is applied
- A post primary antibody solution enhances penetration of the subsequent polymer reagent
- A poly-HRP anti-mouse/rabbit IgG reagent localizes the primary antibody
- The substrate chromogen, 3,3'-diaminobenzidine (DAB), visualizes the complex via a brown precipitate
- Hematoxylin (blue) counterstaining allows the visualization of cell nuclei.

Using Bond Polymer Refine Detection in combination with the automated BOND-III system reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagent Provided

Estrogen Receptor Clone 6F11 is a mouse anti-human monoclonal antibody produced as a tissue culture supernatant, and supplied in Tris buffered saline with carrier protein, containing 0.35% ProClin™ 950 as a preservative.

Total volume = 7 mL (PA0151); 30 mL (PA0009).

Clone

6F11.

Immunogen

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

Specificity

Human estrogen receptor.

Subclass

IgG1.

Total Protein Concentration

Approx 10 mg/mL.

Antibody Concentration

Greater than or equal to 0.88 mg/L as determined by ELISA.

Antibody Development

Estrogen Receptor Clone 6F11 was raised against recombinant ER protein derived from the mRNA of MCF-7 cells. Balb/c mice were immunized with the resulting (His) 6-ER recombinant antigen. Screening was conducted by ELISA, with ELISA positive supernatants tested in IHC on formalin-fixed, paraffin-embedded sections of breast carcinoma of known receptor status. Colonies demonstrating positive immunohistochemical staining were cloned by limiting dilution.

Dilution and Mixing

Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is optimally diluted for use on the automated BOND-III system in combination with Bond Polymer Refine Detection. Further dilution may result in loss of antigen staining. The user must validate any such change. 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. Refer to "Using BOND Reagents" in your BOND user documentation.

Materials Required But Not Provided

Refer to "Using BOND Reagents" in your BOND user documentation for a complete list of materials required for specimen treatment and immunohistochemical staining using the automated BOND-III system.

Storage and Stability

Store at 2–8 °C. Do not use after the expiration date indicated on the container label.

The signs indicating contamination and/or instability of Estrogen Receptor Clone 6F11 are: turbidity of the solution, odor development, and presence of precipitate.

Return to 2–8 °C immediately after use.

Storage conditions other than those specified above must be verified by the user.

Specimen Preparation

All specimens must be prepared to preserve the tissue for immunohistochemical staining.

Standard methods of tissue processing should be used for all specimens.

It is recommended that tissues are prepared in formalin-based fixatives and are routinely processed and paraffin-embedded. For example, resection specimens should be blocked into a thickness of 3–4 mm and fixed for 18–24 hours in 10% neutral-buffered formalin. The tissues should then be dehydrated in a series of alcohols and cleared through xylene, followed by impregnation with molten paraffin wax, held at no more than 60 °C. Tissue specimens should be sectioned between 3–5 µm.

To preserve antigenicity, tissue sections mounted on slides should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C). Following sectioning, it is recommended that slides are incubated at 60 °C for one hour to assist with adherence.

In the USA, the Clinical Laboratory Improvement Act of 1988 requires in 42 CFR 493.1259(b) that "The laboratory must retain stained slides for at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination".

Precautions

- This product is intended for *in vitro* diagnostic use.
- The concentration of ProClin™ 950 is 0.35%. It contains the active ingredient 2-methyl-4-isothiazolin-3-one, and may cause irritation to the skin, eyes, mucous membranes and upper respiratory tract. Wear disposable gloves when handling reagents.
- To obtain a copy of the Material Safety Data Sheet contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems' Web site, www.LeicaBiosystems.com.
- 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 or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
- Consult Federal, State or local regulations for disposal of any potentially toxic components.
- Minimize microbial contamination of reagents or an increase in non-specific staining may occur.
- Retrieval, incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.

Instructions for Use

Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ was developed for use on the automated BOND-III system in combination with Bond Polymer Refine Detection. The recommended staining protocol for Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is IHC Protocol F. Heat induced epitope retrieval is recommended using Bond Epitope Retrieval Solution 1 for 20 minutes.

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-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. The recommended positive control tissue for use with Estrogen Receptor Clone 6F11 is a weakly positive breast carcinoma.

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. The recommended negative control tissue for use with Estrogen Receptor Clone 6F11 is tonsil (endothelium).

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*. 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 (e.g. 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. Normal mouse sera diluted to the same concentration as the primary antibody may be used as a negative control reagent.

Refer to "Using BOND Reagents" in your BOND user documentation.

Staining Procedure (BOND-III System)

1. On the BOND-III system, ensure the bulk and hazardous waste containers have enough capacity to perform the required staining runs.
2. Ensure there is adequate alcohol, distilled or de-ionized water, Bond Dewax Solution (supplied as ready-to-use), Bond Epitope Retrieval Solution 1 (supplied as ready-to-use) and Bond Wash Solution (supplied as 10X concentrate) in the bulk reagent containers to perform the required staining runs.
3. Ensure that a clean BOND Mixing Station is installed.
4. Turn on the BOND-III fully automated, advanced staining system.
5. Turn on the PC attached to the BOND-III fully automated, advanced staining system.
6. Open the BOND-III software.
7. For a new DS9800 Bond Polymer Refine Detection and Ready-To-Use Primary Antibodies for Bond PA0151 (7 mL) / PA0009 (30 mL), scan the reagent barcodes with the handheld scanner to enter the system into the BOND-III reagent inventory.
8. Go to the Slide setup screen and click Add case.
9. Enter details for the first case. Ensure the dispense volume is set to 150 μ L and the preparation protocol is *Dewax. Click OK.
10. With the case highlighted in the Slide setup screen click Add slide.
11. First, add patient test slides. Ensure tissue type is set to Test tissue.
12. Confirm the dispense volume is 150 μ L and the preparation protocol is *Dewax.
13. Select staining mode values Single.
14. Select process IHC.
15. Select *Estrogen Receptor Clone 6F11 from the marker list. The Protocols tab defaults to the correct staining protocol (*IHC Protocol F) and HIER protocol (*HIER 20 min with ER1).
16. Click Add slide. The test slide is created.
17. Repeat steps 9 to 17 until all cases and test slides have been created.
18. Print slide labels and label slides appropriately.
19. Open the lids of all BOND-III containers and load the reagent tray onto the BOND System.
20. Place slides onto the slide tray. Apply BOND Covertile for each test slide.
21. Load the slide tray onto the BOND-III and press the Load/Unload button.
22. Confirm that the slides have been scanned and click the Run (Play) button on the System status screen.
23. Ensure that the tray indicator field displays Proc (OK) and batch number and finish time are displayed.
24. When the run is completed press the Load/Unload button and remove the slide trays from the BOND-III.
25. Remove Covertiles and rinse the slides in de-ionized water.
26. Dehydrate, clear and mount sections.

Troubleshooting

Refer to reference 3 for remedial action.

Contact your local distributor or the regional office of Leica Biosystems to report unusual staining.

Interpretation of Staining

Positive Tissue Control

The positive tissue control stained with Estrogen Receptor Clone 6F11 should be examined first to ascertain that all reagents are functioning properly. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Control

The 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. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells/cellular components. If specific staining (false positive staining) occurs in the negative external tissue control, results with the patient specimen should be considered invalid.

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.

Patient Tissue

Examine patient specimens stained with Estrogen Receptor Clone 6F11 last. The staining pattern of Estrogen Receptor Clone 6F11 is nuclear. 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.

Cautionary Note: Staining may also be present in stromal cells, endothelial cells, lymphocytes and other tissue elements. Interpretation should be assessed in the context of the sample being assessed.

Assay Interpretation

For the determination of estrogen receptor expression, only a nuclear staining pattern should be evaluated. A pathologist using a bright-field microscope should perform slide evaluation. For evaluation of the immunohistochemical staining and scoring, an objective of 10x magnification is appropriate. The use of 20-40x objective magnification should be used in the conformation of the score. Cytoplasmic staining should be considered as non-specific staining and is not to be included in the assessment.

The American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Arch Pathol Lab Med. 2010; 134: e48–e72), should be used for assay interpretation.

Specifically, evaluation is defined as:

- Positive for ER if finding of $\geq 1\%$ of tumor cell nuclei are immunoreactive.
- Negative for ER if finding of $< 1\%$ of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER (positive intrinsic controls are seen).
- Uninterpretable for ER if finding that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining.

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.
- Excessive or incomplete counterstaining may compromise proper interpretation of results.
- The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents and methods to interpret all of the steps used to prepare and interpret the final IHC preparation.
- The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections or cytologic preparation. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
- This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit non-specific staining with horseradish peroxidase.
- Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues.
- Normal/non-immune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
- False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g. liver, breast, brain, kidney) depending on the type of immunostain used.

Product Specific Limitations

Estrogen Receptor Clone 6F11 has been optimized at Leica Biosystems for use with Bond Polymer Refine Detection and BOND ancillary reagents. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances. The protocol times may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement, and must be determined empirically. Negative reagent controls should be used when optimizing retrieval conditions and protocol times.

Performance Characteristics

Immunoreactivity

Table 1: Reactivity of Estrogen Receptor Clone 6F11 on Normal Tissues

Tissue	Number of Cases	Description of Staining	Staining Intensity (0-3+)
Adrenal	3	No staining of tissue elements	0
Brain, Cerebellum	3	No staining of tissue elements	0
Brain, Cerebrum	3	No staining of tissue elements	0
Breast	3	Duct nuclei in 2/3 tissues	3+
Cervix	3	Variable nuclei staining in the stratified squamous epithelium of the ectocervix, cervical stromal cells and glandular tissue	1-2+
Colon	3	No staining of tissue elements	0
Esophagus	3	No staining of tissue elements	0
Heart	3	No staining of tissue elements	0
Kidney	3	No staining of tissue elements	0
Liver	3	No staining of tissue elements	0
Lung	3	No staining of tissue elements	0
Mesothelial cells	1	No staining of tissue elements	0
Ovary	3	Faint variable nuclear staining of a small percentage of ovarian stromal cells. Positive nuclear staining of the <i>theca externa</i> branching into the ovarian stroma in a percentage of ovarian follicles.	1-2+
Pancreas	3	No staining of tissue elements	0
Peripheral nerve	3	No staining of tissue elements	0
Pituitary	3	No staining of tissue elements	0
Prostate	3	No staining of tissue elements	0
Salivary/Submandibular Gland	3	No staining of tissue elements	0
Skeletal Muscle	3	No staining of tissue elements	0
Skin	3	No staining of tissue elements	0
Small Intestine	3	No staining of tissue elements	0
Spleen	3	No staining of tissue elements	0
Stomach	3	No staining of tissue elements	0
Testis	3	No staining of tissue elements	0
Thymus	2	No staining of tissue elements	0
Thyroid	3	No staining of tissue elements	0
Tonsil	3	No staining of tissue elements	0
Uterus	3	Variable nuclei staining of endometrial/myometrial stromal cells	1+
Bone Marrow	3	No staining of tissue elements	0

Key to Staining Intensity:

- 0 - Negative
- 1+ - Weak
- 2+ - Moderate
- 3+ - Strong

Normal Tissues

Estrogen Receptor Clone 6F11 detects the estrogen receptor alpha antigen in the nuclei of cells that express high levels of ER, a proportion of endometrial, ovarian and myometrial cells, and normal breast ductal cells. Staining may also be present in stromal cells, endothelial cells, lymphocytes and other tissue elements.

Clinical Outcome Study

The Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ was tested in an independent clinical outcome study. In summary, the study used a retrospective Calgary-based patient cohort (n=532) composed of breast cancer patients diagnosed between 1985 and 2000, who were treated with primary adjuvant tamoxifen regardless of their ER and PR status. This cohort possesses several unique characteristics that lend to this study, including: it has greater than 5 years of follow-up; it was enriched for events to increase its statistical power; it contains ER negative patients so as to remove treatment selection bias.

To assess differences between methods in the study, the following statistical methods were evaluated and the outcomes described:

1. The Cohen's Kappa statistic to quantify the ease of reproducibility of Allred scoring method (Inter- and Intra-Observer).

Results indicated that Inter-observer kappa for the Leica platform showed almost perfect agreement for ER, with $\kappa=0.67$ between Observers 1 and 2, $\kappa=0.75$ between Observers 1 and 3, and a $\kappa=0.83$ between Observers 2 and 3. Slides were also rescored by Observer 1 three months after the original scoring and intra-observer kappa was calculated with almost perfect agreement of $\kappa=0.91$.

2. Univariate Kaplan-Meier and Multivariate Cox survival analysis using the Allred cutpoint for hormone receptor positivity to dichotomize patients into survival groups.

The univariate outcome is shown in figure 1.

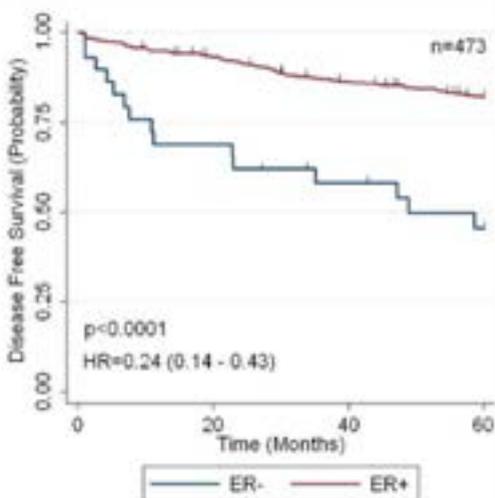


Figure 1: Univariate Analysis for the Leica test Device

Multivariate Cox models were analyzed along with lymph node status, tumour grade, tumour size and HER2 status. The model is shown in figure 2.

	Leica Device (n=363)		
	HR	95% CI	p-value
ER Status	0.39	(0.19 – 0.78)	0.008
Lymph Node Status	3.18	(1.88 – 5.37)	<0.001
Tumor Grade	3.15	(1.84 – 5.38)	<0.001
Tumor Size	1.67	(0.94 – 2.98)	0.083
HER2 Status	1.13	(0.38 – 3.33)	0.823

Figure 2: Multivariate model for the Leica test Device

3. Measures of test performance; sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were calculated. For these calculations, the ligand-binding assay (LBA) was used as the gold standard (figure 3). Also, the Leica assay and the LBA using progression on tamoxifen as the gold standard (figure 4) were calculated.

In this study, each measured test performance can be defined as:

Sensitivity is defined as the proportion of positive subjects correctly identified by the test.
Specificity is defined as the proportion of negative subjects correctly identified by the test.
PPV is defined as the proportion of subjects with a positive test result who were correctly diagnosed.
NPV is defined as the proportion of subjects with a negative test result who were correctly diagnosed.

Figures 3 and 4 show the results obtained.

	Leica Device
Sensitivity	0.97
Specificity	0.44
PPV	0.96
NPV	0.70

Figure 3: Measure of test performance (LBA = Gold Standard)

	Leica Device	LBA
Sensitivity	0.96	0.96
Specificity	0.16	0.16
PPV	0.82	0.84
NPV	0.52	0.44

Figure 4: Measure of test performance (Progression = Gold Standard)

Results from this study show that the test device, the Estrogen Receptor Clone 6F11 has excellent correlation in identification of the optimal clinical assay for the determination of endocrine treatment response in breast cancer.

Reproducibility Study

Reproducibility was performed on the BOND-III system using formalin-fixed paraffin-embedded tissue micro array sections of invasive breast tumor and whole tissue sections of invasive breast tumour.

BOND-III Precision Results

Within Run Precision Study (intra assay – single instrument)

Testing was conducted on one Slide Staining Assembly (SSA) using 108 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, three times, over three different days. Results are as indicated below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	81	0
	Negative	0	27
Overall Percent Agreement (95% CI)		100 (97.26-100.00)	
Positive Percent Agreement (95% CI)		100 (96.37-100)	
Negative Percent Agreement (95% CI)		100 (89.50-100)	

Within Instrument Precision Study (inter assay - single Instrument)

Testing was conducted on three SSA's using 321 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, three times, over three different days. Results are as indicated below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	241	0
	Negative	0	80
Overall Percent Agreement (95% CI)		100 (99.07-100.00)	
Positive Percent Agreement (95% CI)		100 (98.76-100)	
Negative Percent Agreement (95% CI)		100 (96.32-100)	

Between Run Precision Study (inter assay - day-to-day - single instrument)

Testing was conducted on one SSA using 180 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, five times, over five different days, performed over a twenty (20) day period. Results are as indicated below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	135	0
	Negative	0	45
Overall Percent Agreement (95% CI)		100 (98.35-100.00)	
Positive Percent Agreement (95% CI)		100 (97.81-100)	
Negative Percent Agreement (95% CI)		100 (93.56-100)	

Between Laboratory Precision Study (site-to-site - inter assay - multiple instruments)

Testing was conducted on one SSA using 101 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, at 3 investigational sites. Results are as indicated below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	78	0
	Negative	0	23
Overall Percent Agreement (95% CI)		100 (97.08-100.00)	
Positive Percent Agreement (95% CI)		100 (96.23-100)	
Negative Percent Agreement (95% CI)		100 (87.79-100)	

Lot to Lot Precision Study

Testing was conducted on one SSA using 100 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, using three (3) independently manufactured reagent lots of the test device. Results are as indicated below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	75	0
	Negative	0	25
Overall Percent Agreement (95% CI)		100 (97.05-100.00)	
Positive Percent Agreement (95% CI)		100 (96.08-100)	
Negative Percent Agreement (95% CI)		100 (88.71-100)	

Between Observer Precision Study

Between observer precision testing was evaluated between 3 observers at 1 investigational site (Site A). Twenty (20) whole section breast cancer cases consisting of 5x Positive (Strong Intensity Expression) Profile Breast Carcinoma cases, 5x Positive (Medium Intensity Expression) Profile Breast Carcinoma cases, 5x Positive (Weak Intensity Expression) Profile Breast Carcinoma cases and 5x Negative Profile Breast Carcinoma were used.

Between observer agreement between Observer 1 and Observer 2 was 95% (19/20).

Between observer agreement between Observer 1 and Observer 3 was 95% (19/20).

Between observer agreement between Observer 2 and Observer 3 was 100% (20/20).

Between observer agreement between Observer 1, Observer 2 and Observer 3 was 98.33% (59/60).

Inter-Site Reproducibility

Inter-site reproducibility testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III was evaluated at 3 investigational sites on whole tissue sections. The test cohort consisted of 18 cases. Testing was performed over a span of 5 non-consecutive days, with each site staining a full set of cases on each day. This provided 9 replicates of 18 cases.

Results for Inter-Site reproducibility are presented as overall (3 sites combined), site to site (2 sites) and by single site alone (within-site).

Overall (3 sites combined)

Inter-Site agreement between Site A, Site B and Site C was 94.44 % (95% confidence interval of 89.72% to 97.43%) as shown in the table below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	96	6
	Negative	3	57
Overall Percent Agreement (95% CI)		94.44% (89.72-97.43)	
Positive Percent Agreement (95% CI)		96.97% (91.40-99.37)	
Negative Percent Agreement (95% CI)		90.48% (80.41-96.42)	

Inter-site agreement between Site A and Site B was 88.89% (48/54).

Inter-site agreement between Site A and Site C was 92.59% (50/54).

Inter-site agreement between Site B and Site C was 92.59% (50/54).

Within-site agreement for Site A alone was 94.44% (52/54).

Within-site agreement for Site B alone was 98.15% (53/54).

Within-site agreement for Site C alone was 98.15% (53/54).

Lot to Lot Precision Study

Lot to lot precision studies were evaluated at a single investigational site on whole tissue sections. The test cohort consisted of 18 cases. Testing was conducted using three (3) independently manufactured reagent lots. This format provided 3 replicates of each slide (one for each reagent lot).

Results are presented as below.

		Positive	Negative
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the BOND-III	Positive	32	1
	Negative	1	20
Overall Percent Agreement (95% CI)		96.30% (87.25-99.55)	
Positive Percent Agreement (95% CI)		96.97% (84.24-99.92)	
Negative Percent Agreement (95% CI)		95.24% (76.18-99.88)	

Published Immunoreactivity

Characterization of Estrogen Receptor Clone 6F11 during antibody development included a comparative evaluation of a series of 55 sequential breast carcinomas. The tissues evaluated were routinely processed formalin-fixed, paraffin-embedded specimens stained using both Estrogen Receptor Clone 6F11 and ER (1D5). There was an observed concordance of staining for 50/55 cases⁸.

Estrogen receptor status was evaluated in 592 cases using routinely prepared paraffin-embedded tissue samples from primary breast carcinomas with Estrogen Receptor Clone 6F11 and ER (1D5). Overall, ER (1D5) and Estrogen Receptor Clone 6F11 showed a 97.5% concordance rate¹⁶.

Further Information

Further information on immunostaining with BOND reagents, under the headings Principle of the Procedure, Materials Required, Specimen Preparation, Quality Control, Assay Verification, Interpretation of Staining, Key to Symbols on Labels, and General Limitations can be found in "Using BOND Reagents" in your BOND user documentation.

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