



# Novocastra™ Liquid Mouse Monoclonal Antibody Nitric Oxide Synthase-1

**Product Code: NCL-L-NOS-1**

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## **Instructions for Use**

Please read before using this product.

**Check the integrity of the packaging before use.**

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**Rx Only**



# Novocastra™ Liquid Mouse Monoclonal Antibody

## Nitric Oxide Synthase-1

### Product Code: NCL-L-NOS-1

#### Intended Use

For *in vitro* diagnostic use.

NCL-L-NOS-1 is intended for the qualitative identification by light microscopy of human nitric oxide synthase-1 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.

**NCL-L-NOS-1 is recommended for the assessment of nitric oxide synthase-1 protein expression in normal and neoplastic tissues.**

#### Summary and Explanation

The first immunohistochemical technique was reported by Nakane and Pierce.<sup>1</sup> Since then many developments have occurred, leading to increased sensitivity over earlier techniques. A recent development has been the use of polymeric labeling. This technology has been applied to both primary antibodies<sup>2</sup> and detection systems. The Novolink™ Polymer Detection Systems utilize a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. Therefore, the problem of non-specific staining that can occur with Streptavidin/Biotin detection systems due to endogenous biotin does not occur.

#### 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.

#### Reagent

NCL-L-NOS-1 is a liquid tissue culture supernatant containing sodium azide as a preservative.

#### Clone

NOS-125

#### Immunogen

Prokaryotic recombinant protein corresponding to a portion of the C-terminus of the mature human nitric oxide synthase-1 protein.

#### Specificity

Human nitric oxide synthase-1. Also known as neuronal NOS or nNOS.

#### 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 27 mg/L as determined by ELISA. Refer to vial label for lot specific Ig concentration.

#### 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.<sup>3</sup> 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.

#### 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.

The signs indicating contamination and/or instability of NCL-L-NOS-1 are: turbidity of the solution, odor development, and presence of precipitate.

#### Specimen Preparation

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

## 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:** 1:100 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.

## Materials Provided

See Reagent.

## Materials Required But Not Provided

See Novolink™ Polymer Detection Systems Instructions for Use.

## 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>4</sup>

Recommended positive control tissue is colon.

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 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>5</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-NOS-1 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 NOS-125 detected the NOS-1 protein in neural elements of the gastrointestinal tract and neurons in the central nervous system. (Total number of normal cases evaluated = 43).

### Abnormal Tissues

Clone NOS-125 stained 1/1 ganglioneuroma.

No staining was detected in brain tumors (0/2), tumors of the esophagus (0/2), a tumor of the larynx (0/1), a tumor of the thymus (0/1), thyroid tumors (0/3), breast tumors (0/2), stomach tumors (0/2), tumors of the tongue (0/2), lung tumors (0/4), metastatic tumors of unknown origin (0/2), liver tumors (0/4), kidney tumors (0/2), ovarian tumors (0/4), tumors of the cervix (0/2), testicular tumors (0/2), colonic tumors (0/2), tumors of the rectum (0/2) and skin tumors (0/2). (Total number of tumor cases evaluated = 43).

## 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>6</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.

## Performance Characteristics

The performance of NCL-L-NOS-1 has been validated on a range of normal and abnormal tissues. See Results Expected.

## Bibliography - General

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## Amendments to Previous Issue

Antibody Concentration.

## Date of Issue

20 February 2019





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