Novocastra™ Liquid Rabbit Polyclonal Antibody Serotonin

Product Code: NCL-L-SEROTp

Check the integrity of the packaging before use.

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Intended Use
For in vitro diagnostic use.

NCL-L-SEROTp is intended for the qualitative identification by light microscopy of human serotonin protein 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-SEROTp is recommended for the assessment of serotonin protein expression in normal and neoplastic tissues

Summary and Explanation
The first immunohistochemical technique was reported by Nakane and Pierce. 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 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-SEROTp is a liquid immunoglobulin fraction purified from rabbit serum diluted in PBS with 1% BSA containing sodium azide. Traces of crossreactive antibodies have been removed by solid-phase absorption with human plasma and cow serum. Volume as indicated on vial label.

Clone
Polyclonal

Immunogen
Serotonin/creatinine sulfate complex conjugated to bovine serum albumin.

Specificity
Human serotonin (5-hydroxytryptamine, 5-HT).

Ig Class
Not applicable.

Total Protein Concentration
Refer to vial label for lot specific total protein concentration.

Antibody Concentration
Not applicable.

Warnings and Precautions
This reagent has been prepared from purified rabbit serum. 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

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.

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-SEROTp 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.


Suggested dilution: 1:500 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

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. Recommended positive control tissue is bowel.

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 skin.

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 (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-SEROTp 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

Rabbit polyclonal serotonin detected the serotonin protein in the cytoplasm of neuroendocrine cells within a variety of normal tissues evaluated, including pancreas, cerebellum, basal ganglia and placenta basal plate. Staining was observed in scattered neuroendocrine cells within ileum, cecum, colon and rectum. (Total number of normal cases evaluated = 73).

Abnormal Tissues

Rabbit polyclonal serotonin stained 4/42 pancreatic ductal adenocarcinomas, 1/6 metastatic ductal carcinomas and 2/10 pancreatic inflammation.

No staining was observed in pancreatic adenosquamous carcinomas (0/3), an islet cell carcinoma (0/1), benign islet cell tumors (0/10), pancreatic hyperplasias (0/2), brain tumors (0/2), esophageal tumors (0/2), a laryngeal tumor (0/1), thymus tumors (0/2), thyroid tumors (0/3), breast tumors (0/2), stomach tumors (0/2), soft tissue tumors (0/2), tongue tumors (0/2), lung tumors (0/4), metastatic tumors of unknown origin (0/2), liver tumors (0/4), renal tumors (0/2), ovarian tumors (0/4), a cervical tumor (0/1), testicular tumors (0/2), colonic tumors (0/2), rectal tumors (0/2) and skin tumors (0/2). (Total number of abnormal cases evaluated = 117).
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 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-SEROTp has been validated on a range of normal and abnormal tissues. See Results Expected.

Bibliography - General


Amendments to Previous Issue

Not applicable

Date of Issue

20 July 2012