



Novocastra™ Liquid Mouse Monoclonal Antibody p57 Protein (Kip2)

Product Code: NCL-L-p57

Leica Biosystems Newcastle Ltd
Balliol Business Park West
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
☎ +44 191 215 4242



Instructions for Use

Please read before using this product.

Check the integrity of the packaging before use.

Novocastra™ Liquid Mouse Monoclonal Antibody p57 Protein (Kip2)

Product Code: NCL-L-p57

Intended Use

For *in vitro* diagnostic use.

NCL-L-p57 is intended for the qualitative identification by light microscopy of human p57 protein, also known as Kip2 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-p57 is recommended for the assessment of hydropic and simple abortions and partial and complete hydatidiform moles.

Summary and Explanation

The first immunohistochemical technique was reported by Nakane and Pierce.¹ 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 antibodies² 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-p57 is a liquid tissue culture supernatant containing sodium azide as a preservative.

Clone

25B2

Immunogen

Prokaryotic recombinant antigen corresponding to a 116 amino acid region of the N-terminus of the p57 protein.

Specificity

Human p57 protein, also known as Kip2 protein.

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 19 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

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.³ 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-p57 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: 1:50 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

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.⁴

Recommended positive control tissue is placenta.

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 skeletal muscle.

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.⁵ 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-p57 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 25B2 detected the p57 protein in the nucleus of Hofbauer cells, trophoblasts and decidua of placenta, fibroblastic cells of Wharton's jelly in the umbilical cord, adrenal cortical cells in the zona glomerulosa, glomerular endothelial cells in the kidney and occasional cells within the seminiferous tubules and endometrial stroma.

Cytoplasmic and nuclear staining was seen in acinar cells in the pancreas and epithelium in the ileum, cecum, gall bladder and cervix. Cytoplasmic staining was seen in ductal epithelium of the parotid gland, macrophages and occasional pneumocytes in lung, with weak staining in ductal epithelial cells in breast, kidney tubules, and epithelia and chondrocytes in bronchus.

Staining was also noted of nerve fibers and ganglia of the gastrointestinal tract, and of neuronal processes and axons of the cerebral cortex, basal ganglia, hippocampus and the granular layer of the cerebellum. (Number of normal cases evaluated = 44).

Abnormal Tissues

Clone 25B2 displayed nuclear staining in 28/31 hydropic abortions, 20/20 simple abortions, 32/34 partial hydatidiform moles and 5/33 complete hydatidiform moles. (Sharifi, et al 2009, Maggiori & Peres 2007).

Staining was also observed in 3/4 papillary carcinomas of the thyroid, 1/2 soft tissue tumors (including 1/1 ganglioneuroma and 0/1 fibromatosis), 1/2 brain tumors (including 1/1 anaplastic astrocytoma and 0/1 choroid plexus papilloma), 2/2 metastatic tumors of unknown origin, and 2/2 renal cell carcinomas. Cytoplasmic staining was observed in 1/2 infiltrating ductal carcinomas of the breast, 1/2 gastric adenocarcinomas, with weak staining in 1/1 cholangiocarcinoma. Focal cytoplasmic staining was observed in 2/2 squamous cell carcinomas of the esophagus, 1/1 squamous cell carcinoma of the larynx, 2/2 squamous cell carcinomas of the tongue and 1/2 squamous cell carcinomas of the cervix. Weak nuclear and/or cytoplasmic staining was observed in 2/4 lung tumors (including 1/1 non-small cell carcinoma, 0/1 adenocarcinoma, 1/1 squamous cell carcinoma and 0/1 large cell carcinoma), 1/1 mucinous cystadenocarcinoma of the ovary and 1/2 adenocarcinomas of the colon. Weak nuclear staining was observed in 1/1 atypical carcinoid of the thymus and 1/2 hepatocellular carcinomas. No staining was observed in testicular seminomas (0/2), adenocarcinomas of the rectum (0/2), a metastatic carcinoma of the liver, a malignant ovarian germ cell tumor, an ovarian serous cystadenocarcinoma, an ovarian clear cell carcinoma, a dermatofibrosarcoma and a squamous cell carcinoma of the skin. (Number of abnormal cases evaluated = 162).

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.⁶ 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-p57 has been validated on a range of normal and abnormal tissues. See Results Expected.

Bibliography - General

1. Nakane PK and Pierce GB. Enzyme labeled antibodies : Preparations and applications for the localization of antigens. *Journal of Histochemistry and Cytochemistry*. 1967; 14:929–931.
2. Tsutsumi Y, Serizawa A and Kawai K. Enhanced polymer one-step staining (EPOS) for proliferating cell nuclear antigen and Ki-67 antigen-applications to intraoperative frozen diagnosis. *Pathology International*. 1995; 45(2):108–115.
3. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, P.A. 1991; 7(9). Order code M29-P.
4. Battifora H. Diagnostic uses of antibodies to keratins: a review and immunohistochemical comparison of seven monoclonal and three polyclonal antibodies. *Progress in Surgical Pathology*. 6:1–15. eds. Fenoglio-Preiser C, Wolff CM, Rilke F. Field & Wood, Inc., Philadelphia.
5. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and pitfalls. *Laboratory Medicine*. 1983; 14:767.
6. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen: a possible source of error in immunohistochemistry. *American Journal of Clinical Pathology*. 1980; 73:626.
7. Kanthan R, Fried I, Rueckl T, et al. Expression of cell cycle proteins in male breast carcinoma. *World Journal of Surgical Oncology* 2010; 8:10.
8. Sharifi N, Sadeghian MH, Ayatollahi H, et al. Validity of P57kip2 immunohistochemical marker in differential diagnosis of molar pregnancy. *Journal of the Turkish-German Gynecological Association* 2009;10:39-42.
9. Maggiori MS and Peres LC. Morphological, immunohistochemical and chromosome in situ hybridization in the differential diagnosis of hydatidiform mole and hydropic abortion. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 2007; 135: 170-176.

Amendments to Previous Issue

Not applicable.

Date of Issue

13 August 2014

Leica Biosystems Newcastle Ltd
Balliol Business Park West
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
☎ +44 191 215 4242

