

HƯỚNG DẪN SỬ DỤNG BẢN GỐC

Tên cơ sở công bố lưu hành:

Công ty TNHH DH Holding Việt Nam

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Xác nhận tài liệu đính kèm.

Novocastra™ Lyophilized Mouse Monoclonal Antibody Utrophin (N-terminus)

Product Code: NCL-DRP2

Intended Use

For in vitro diagnostic use.

NCL-DRP2 is intended for the qualitative identification by light microscopy of Utrophin (N-terminus) by immunohistochemistry. 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

DRP3/20C5

Immunogen

Fusion protein containing the first 261 amino acids of the published DMDL gene sequence.

Specificity

Amino terminal domain of the human homolog of human dystrophin, utrophin (also known as dystrophin related protein or "DRP"). Also crossreacts with utrophin in sections of muscle from rat and dog. Other animals species have not been tested.

Reagent Composition

NCL-DRP2 is a lyophilized tissue culture supernatant containing sodium azide as a preservative. The user is required to reconstitute the contents of the vial with the correct volume of sterile distilled water as indicated on the vial label.

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 126.0 mg/L as determined by ELISA. Refer to vial label for batch specific Ig concentration.

Recommendations On Use

Immunohistochemistry (see **Methodology**) on frozen sections. Suggested dilution: 1:2–1:10 for 60 minutes at 25 °C. This is provided as a guide and users should determine their own optimal working dilutions.

Storage and Stability

Store unopened antibody at 2–8 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Do not use after expiration date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 2–8 °C. For long term storage, it is recommended that aliquots of the reconstituted antibody are stored frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use. Return to 2–8 °C immediately after use. Storage conditions other than those specified above must be verified by the user.

Specimen Preparation

Freeze specimen tissue blocks in isopentane chilled in liquid nitrogen (see Warnings and Precautions). The specimens do not require further fixation but should be embedded in OCT™ compound (Sakura, Product No. Tissue-Tek 4583).

Warnings and Precautions

Novocastra Lyophilised Antibodies

Contains a mixture of:
Sodium
Azide (<10%),
Benzylpenicillin Sodium
(<10%), Streptomycin
Sulphate (<10%), Signal
words: Danger.

H302: Harmful if swallowed.
H317: May cause an allergic
skin reaction.
H334: May cause allergy or
asthma symptoms or breathing
difficulties if inhaled.
H411: Toxic to aquatic life with
long lasting effects.
EUH032: Contact with acids
liberates very toxic gas.

P261: Avoid breathing dust/fumes/gas/mist/vapours/spray.
P264: Wash hands thoroughly after handling.
P270: Do not eat, drink or smoke when using this product.
P272: Contaminated work clothing should not be allowed out of the
workplace.
P273: Avoid release to the environment.
P280: Wear protective gloves/protective clothing/eye protection/
face protection.
P284: In case of inadequate ventilation wear respiratory protection.
P301+312: IF SWALLOWED: Call a POISON CENTER/doctor/ if
you feel unwell.
P302+352: IF ON SKIN: Wash with plenty of soap and water.
P304+340: IF INHALED: Remove person to fresh air and keep
comfortable for breathing.
P330: Rinse mouth.
P333+313: If skin irritation or rash occurs: Get medical advice/
attention.
P342+311: If experiencing respiratory symptoms: Call a POISON
CENTER/doctor.
P362+364: Take off contaminated clothing and wash it before reuse.
P391: Collect spillage.
P501: Dispose of contents/container to hazardous or special waste
collection point.

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. 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.

Liquid nitrogen due to its excessively cold temperature causes burns and protective clothing, including gloves and visor, should be used when handling. Use in a well ventilated area.

Isopentane is highly flammable and harmful by ingestion and inhalation. It is also irritating to skin and eyes, and is a narcotic in high concentration.

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 frozen 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.²

Recommended positive control tissue is the mid-section of rat soleus muscle where neuromuscular junctions can be located utilising double labelling with fluorescent alpha-bungarotoxin to identify the specific location of utrophin.

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 has not been evaluated.

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.³ 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-DRP2 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 DRP3/20C5 detects the amino terminal domain of the chromosome-6 encoded homolog of human dystrophin, utrophin, (also known as dystrophin related protein or "DRP"). Utrophin labelling is restricted to neuromuscular junctions in normal adult human muscle and not the general periphery of the muscle fibers. Blood vessels, capillaries and nerves are also labelled. Fibers from dystrophin-deficient muscle (for example, from DMD or BMD patients or carriers) show a more extensive labelling of muscle fiber membranes so that almost all fibers show some labelling. Utrophin is also expressed at the membranes of small, regenerating fibers that also label with antibodies to proteins like neonatal myosin.

Abnormal Tissues

Clone DRP3/20C5 has been used in immunohistochemical studies of more than 300 patients to identify expression of the dystrophin-related protein, utrophin.

NCL-DRP2 is recommended for the identification of human Utrophin (N-terminus) by immunohistochemistry.

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 artefacts, 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.⁴ 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.

Bibliography - General

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5. Bornemann A and Anderson LVB. Diagnostic protein expression in human muscle biopsies. Brain Pathology. 2000; 10:193-214.
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Amendments to Previous Issue

Not Applicable.

Date of Issue

17 October 2018

Immunohistochemistry methodology for using Novocastra™ antibodies on frozen muscle tissue.

Reagents required but not supplied

1. Standard solvents used in immunohistochemistry.
2. 50 mM Tris-buffered saline (TBS) pH 7.6.
3. Antibody diluent - normal serum optimally diluted in TBS.
4. Normal serum from the species in which the secondary antibody is raised.
5. Secondary peroxidase-conjugated antibody - use as recommended by manufacturer.
6. 3,3' Diaminobenzidine tetrahydrochloride (DAB) - prepare and use as recommended by manufacturer.
7. Mounting medium - use as recommended by manufacturer.

Equipment required but not supplied

1. Incubator set to 25 °C.
2. General immunohistochemistry laboratory equipment.
3. Electric fan for air drying slides.

Antigen retrieval solutions (see Recommendations on Use)

Not applicable to frozen sections.

Methodology

Prior to undertaking this methodology, users must be trained in immunohistochemical techniques. Users should determine optimal dilutions for antibodies. Unless indicated, all steps are performed at 25 °C.

1. Cut and mount 4–10 µm sections on slides coated with a suitable tissue adhesive and air dry for at least one hour.
2. Incubate sections with optimally diluted primary antibody (see Recommendations on Use).
3. Wash in TBS buffer for 2 x 5 minutes with gentle rocking.
4. Incubate sections in appropriate peroxidase-conjugated secondary antibody.
5. Wash in TBS buffer for 2 x 5 minutes with gentle rocking.
6. Incubate the slides in DAB.
7. Rinse the slides in clean water.
8. Dehydrate, clear and mount sections.

Amendments to Previous Issue

Not applicable.

Date of Issue

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