Novocastra™ Lyophilized Mouse Monoclonal Antibody Dysferlin

Product Code: NCL-Hamlet

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Reactive with the dysferlin molecule in human skeletal muscle. Reacts with mouse, rat, rabbit, hamster, pig and dog muscle but not with chicken. Other species not tested. Also present in many non-muscle tissues. Severely reduced intensity of labelling in the SJL mouse.
Clone	Ham1/7B6
Ig Class	IgG1
Antigen Used for Immunizations	Synthetic peptide containing amino acids 1999–2016 of the human dysferlin molecule (within exon 53).
Hybridoma Partner	Mouse myeloma (X63.Ag8.653) x CD1.
Preparation	Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Yes. Optimum fixative, Acetone/Methanol (1:1), 4 minutes at room temperature.
Effective on Paraffin Wax Embedded Tissue	Yes
Recommendations on Use	Immunohistochemistry: Typical working dilution on fixed frozen sections 1:20–1:40. Indirect immunoperoxidase technique (see overleaf). Typical working dilution on fixed paraffin sections 1:20–1:40. The high temperature antigen unmasking technique may improve staining in some cases (see attached). 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: See Anderson L V B et al.. Human Molecular Genetics (1999).
Positive Controls	Immunohistochemistry: Normal human skeletal muscle. Western Blotting: Skeletal muscle.
Staining Pattern	Membrane staining of muscle fibers (also shows slight cytoplasmic localization in a fiber-type mosaic).
Storage and Stability	Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are 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.
General Overview	Dysferlin is the protein product of the 2p13 gene that is defective in Limb-Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi Myopathy (MM). Dysferlin is normally localized to the muscle plasma membrane. NCL-Hamlet is of use in research studies of LGMD2B and MM. Labelling with an antibody to beta-spectrin, to monitor membrane integrity, is an essential immunohistochemical control.
General References	Anderson L V B, Davison K, Moss J A, et al.. Human Molecular Genetics. 8: 855–861 (1999). Anderson L V B and Davison K. American Journal of Pathology. 154 (4): 1017–1022 (1999). Bittner R E, Anderson L V B, Burkhardt E, et al.. Nature Genetics. 23: 141–142 (1999). Weiler T, Bashir R, Anderson L V B, et al.. Human Molecular Genetics. 8: 871–877 (1999).

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

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BIOSYSTEMS

Instructions for Use

Protocol for Immunohistochemical use of the following Monoclonal Antibodies: NCL-alpha-ACT, NCL-a-SARC, NCL-b-SARC, NCL-d-SARC, NCL-g-SARC, NCL-b-DG, NCL-MHCd, NCL-MHCf, NCL-MHCn, NCL-MHCs, NCL-SPEC1, NCL-SPEC2, NCL-DRP2, NCL-MEROSIN, NCL-Hamlet and NCL-Hamlet-2.

1. Freeze muscle blocks in isopentane chilled in liquid nitrogen.
2. Cut 4–10 µm sections and air dry on slides coated with tissue adhesive.
3. Slides may be stored below -70 °C wrapped in cling film until required. If stored sections are used, allow sections to equilibriate to 25 °C before unwrapping and proceeding.
4. Apply a 50 µl aliquot of primary antibody to section (unfixed) Use Antibody Diluent RE7133 (where available). Incubate for 1 hour at 25 °C or 37 °C.
   Please note that where NCL-Hamlet and NCL-Hamlet-2 primary antibodies are used, it is recommended that sections are fixed in acetone/methanol (1:1) for 4 minutes at room temperature prior to incubation with the primary antibody.
5. Wash sections in TBS* buffer (pH 7.6) for 3 x 10 minutes.
6. Apply a 50 µL aliquot of labeled secondary antibody (e.g. NCL-GAMP diluted 1:100). Incubate for 1 hour at 25 °C.
7. Wash sections in TBS* buffer (pH 7.6) for 3 x 10 minutes.
8. Mount fluorescent sections in aqueous mountant or visualize peroxidase label (e.g. by exposure to freshly prepared 0.05% w/v diaminobenzidine in TBS* buffer containing 0.1% w/v hydrogen peroxide). Dehydrate, clear and mount peroxidase labeled sections for permanent preparations.

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

BIOSYSTEMS

Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

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Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.