Novocastra™ Lyophilized Mouse Monoclonal Antibody Merosin Laminin Alpha 2 Chain

Product Code: NCL-MEROSIN

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Reacts strongly with laminin α2 chain of merosin in human and rabbit skeletal muscle. No reaction is observed in muscle sections from mouse, rat, dog, chicken, hamster or pig.
Clone	Mer3/22B2
Ig Class	IgG1, kappa
Antigen Used for Immunizations	Purified protein from human placenta.
Hybridoma Partner	Mouse myeloma (X63.Ag8.653).
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 - unfixed.
Effective on Paraffin Wax Embedded Tissue	No
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:50–1:100. 60 minutes primary antibody incubation at 25 °C. Indirect immunoperoxidase technique (see overleaf). Western Blotting: Not recommended.
Positive Controls	Immunohistochemistry: Normal human striated muscle frozen in isopentane chilled in liquid nitrogen.
Staining Pattern	Continuous labelling of the extracellular matrix outside normal muscle fibre membranes.
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	The dystrophin/glycoprotein complex is sited at the muscle membrane. An extracellular member of this complex is alpha-dystroglycan and linked to this, in the extracellular matrix, is laminin. The muscle specific form of laminin, merosin, is composed of three chains: alpha 2, beta 1 and gamma 1. NCL-MEROSIN is suitable for testing tissue from muscle, skin or placenta. This antibody has the sensitive characteristics of an antibody that reacts with the 300 kD fragment of merosin (Sewry et al., 1998). **Important**: For reliable interpretation of merosin laminin alpha 2 chains labelling patterns using tissue sections, the use of a SPECTRIN control is essential.
General References	Sewry C A, Anderson L V B, Bushby K, et al.. Muscle & Nerve. Supplement 7: S109 (1998). Sewry C A, Philpot J, Sorokin L M, et al.. Lancet. 347: 582–584 (1996). Helbling-Leclerc A, Zhang X, Topaloglu H, et al.. Nature Genetics. 11: 216–218 (1995). Philpot J, Sewry C, Pennock J, et al.. Neuromuscular Disorders. 5: 301–305 (1995). Sewry C A, Philpot J, Mahony D, et al.. Neuromuscular Disorders. 5: 307–316 (1995). Sunada Y, Edgar T S, Lotz B P, et al.. Neurology. 45: 2084–2089 (1995). Voit T, Sewry C A, Meyer K, et al.. Neuropediatrics. 26: 148–155 (1995). Voit T, Fardeau M and Tomé F M S. Neuropediatrics. 25: 332–333 (1994). Tomé F M S, Evangelista T, Leclerc A, et al.. C. R. Acad. Sci. Paris. 317: 351–357 (1994).

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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).*

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