Novocastra™ Lyophilized Mouse Monoclonal Antibody Spectrin (recommended for human use)

Product Code: NCL-SPEC1

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Beta chain of spectrin in human red blood cells and muscle.
Clone	RBC2/3D5
Ig Class	IgG2b
Antigen Used for Immunizations	Human red blood cell membrane “ghosts”.
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 - unfixed.
Effective on Paraffin Wax Embedded Tissue	No
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100. 60 minutes primary antibody incubation at 25 °C. Indirect immunoperoxidase technique (see overleaf). Western Blotting: Typical working dilution 1:25–1:50. Electron microscopy gold: Light fixation with 2% formaldehyde + 0.001% glutaraldehyde for 1 hour, 2.3 M sucrose used as cryoprotectant is recommended. Typical working dilution NEAT. 90 minutes primary antibody incubation at 25 °C.
Positive Controls	Immunohistochemistry: Normal human striated muscle frozen in isopentane chilled in liquid nitrogen. Western Blotting: Skeletal muscle.
Staining Pattern	Light microscope: continuous rim of labelling at the periphery of muscle fibres. Western blotting: major band at approximately 253 kD in muscle, 220 kD in red blood cell “ghosts”. (Journal of Biological Chemistry. 265: 20449–20454 (1990)). Electron microscopy gold: close to cytoplasmic face of the plasma membrane of muscle fibres.
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	Spectrin is a cytoskeletal protein which has some structural homology with dystrophin, the protein that is defective in Duchenne and Becker muscular dystrophy. Subtle membrane damage frequently occurs during the excision and freezing of muscle biopsies. Immunohistochemical labelling for spectrin ought to be used to monitor membrane integrity when evaluating proteins such as dystrophin. Fibres that show negative labelling for spectrin are an indication of damage to the muscle being examined.
General References	Sheriffs I N, Rampling D and Smith V V. Journal of Clinical Pathology. 54: 517–520 (2001). Winkelmann J C, Costa F F, Linzie B L, et al.. Journal of Biological Chemistry. 265 (33): 20449–20454 (1990).

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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).*