Novocastra™ Lyophilized Mouse Monoclonal Antibody
Alpha Actinin

Product Code: NCL-alpha-ACT

Intended Use
FOR RESEARCH USE ONLY.

Specificity
Reacts moderately with the isoform of alpha-actinin in human type I slow twitch skeletal muscle fibres and red blood cell membranes. Does not react with alpha-actinin in type IIa or IIb muscle fibres. Also crossreacts strongly with dog, chicken and pig alpha-actinin and weakly with rat alpha-actinin. No reaction is observed with mouse, rabbit and hamster alpha-actinin.

Clone
RBC2/1B6

Ig Class
IgG1

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 1:40–1:80. Indirect immunoperoxidase technique (see overleaf). Western Blotting: Typical working dilution 1:50–1:100.

Positive Controls
Immunohistochemistry: Normal human striated muscle frozen in isopentane chilled in liquid nitrogen.
Western Blotting: Skeletal muscle.

Staining Pattern
Striations localized to the Z disc in longitudinal sections.

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
Alpha-actinin is a rod-like cytoskeletal protein belonging to the same family as spectrin, dystrophin and utrophin. In skeletal muscle, alpha-actinin is located in the Z band/disc and cross-links with F-actin in this region. Muscle tissues show the presence of abundant threadlike particles, known as nemaline bodies, in the myofibres. Electron microscopy studies have shown that the nemaline rods have a lattice structure similar to that of the Z discs and the rods are thought to be lateral polymers of the Z discs.

General References
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 equilibrate 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).