Novocastra™ Lyophilized Mouse Monoclonal Antibody Beta-Dystroglycan

Product Code: NCL-b-DG

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human beta-dystroglycan (43 kD). Also crossreacts strongly with beta-dystroglycan in sections of mouse, rat, rabbit, dog and chicken, hamster and toad muscle. Other animal species not tested.
Clone	43DAG1/8D5
Ig Class	IgG2a
Antigen Used for Immunizations	Synthetic peptide containing 15 of the last 16 amino acids at the extreme C-terminus of the human dystroglycan sequence (PKNMTPYRSPPPYVP-PCOOH).
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 1:50–1:200. 60 minutes primary antibody incubation at 25 °C. Indirect immunoperoxidase technique (see overleaf). Standard ABC technique. Western Blotting: Typical working dilution 1:25–1:50. Electron microscopy gold: Light fixation with 2% formaldehyde + 0.001% glutaraldehyde for 1 hour, 2.3M sucrose used as a cryoprotectant is recommended. Typical working dilution NEAT. 90 minutes primary antibody incubation at 25 °C.
Positive Controls	Immunohistochemistry: Snap frozen normal human striated muscle. Western Blotting - Skeletal muscle. Electron microscopy gold - Normal human striated muscle.
Staining Pattern	Light microscope: Continuous rim of labeling at the periphery of muscle fibers. Electron microscopy gold: close to cytoplasmic face of the plasma membrane of muscle fibers. Western blotting: Band at approximately 43 kD in muscle extracts.
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	In normal skeletal muscle, dystrophin is attached to the muscle membrane via a complex of at least seven proteins (dystrophin associated glycoproteins, DAGs). The biological significance of this dystrophin/glycoprotein complex is not fully understood, but it appears to form an essential linkage between actin on the inside of the muscle fiber and muscle laminin in the basal lamina which surrounds the fiber. Beta-dystroglycan spans the muscle membrane and it has been suggested that it is the member of the complex which binds directly to dystrophin. Labeling of beta-dystroglycan may be reduced where another component eg dystrophin or laminin is directly affected. Labeling with an antibody to beta-spectrin, eg NCL-SPEC1 (recommended for human use), to monitor membrane integrity, is an essential immunohistochemical control in any research performed.
General References	Sheriffs I N, Rampling D and Smith V V. Journal of Clinical Pathology. 54: 517–520 (2001). Cullen M J, Walsh J and Nicholson L V B. Acta Neuropathol. 87: 349–354 (1994). Bewick G S, Nicholson L V B, Young C, et al.. Neuromuscular Disorder. 3: 503–506 (1993).

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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).*