Novocastra™ Lyophilized Mouse Monoclonal Antibody Dystrophin (N-terminus)

Product Code: NCL-DYS3

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Reacts strongly with the amino terminal domain (between amino acids 321 and 494) of human dystrophin. No reactivity with DMD/BMD patients deleted for exons 10 to 12. No crossreaction is observed with mouse (high background only), rat, rabbit, dog, chicken, hamster and pig dystrophin.
Clone	Dy10/12B2
Ig Class	IgG2a
Antigen Used for Immunizations	Fusion protein containing amino acids 67 to 713. Patient immunoreactivity indicates epitope is near exons 10 to 12. Epitope mapping suggests that sequences from amino acids 308 to 351 are involved in antibody binding. This region spans the junction of exons 9 and 10 and the epitope recognised may be part of a hinge region joining the amino domain to the central rod domain.
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 NEAT–1:20. 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: Snap frozen normal human or rat striated muscle. Western Blotting: Skeletal muscle. Electron microscopy gold: Snap frozen normal human or rat striated muscle.
Staining Pattern	Light microscope: continuous rim of labelling at the periphery of muscle fibres. Western blotting: doublet of bands at approximately 400 kD. Electron microscopy gold: close to cytoplasmic face of the plasma membrane.
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	Duchenne muscular dystrophy (DMD) is the most severe of the muscular dystrophies resulting in progressive muscular wasting and death. Dystrophin is the 427 kD protein product of the Duchenne Muscular Dystrophy/Becker Muscular Dystrophy (DMD/BMD) gene located on the X chromosome at position Xp21. Western blotting and immunohistochemistry are the two established methods for use in research studies for the detection of abnormalities of dystrophin expression in muscle biopsies. Important: For reliable interpretation of dystrophin labelling patterns using tissue sections, the use of a SPECTRIN control is essential.
General References	Sheriffs I N, Rampling D and Smith V V. Journal of Clinical Pathology. 54: 517–520 (2001).

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BIOSYSTEMS

Instructions for Use

Protocol for Immunohistochemical Use of Monoclonal Antibodies to Dystrophin; NCL-DYS1, NCL-DYS2 and NCL-DYS3

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 at -70 °C wrapped in cling film until required. If stored sections are used, allow sections to equilibriate to room temperature before unwrapping and proceeding.
4. Apply a 50 µL aliquot of primary antibody to section (unfixed). Incubate for 1 hour at 25 °C or 37 °C.
5. Wash sections 3 x 10 minutes in phosphate buffered saline.
6. Apply a 50 µL aliquot of labelled secondary antibody. Incubate for 1 hour at 25 °C.
7. Wash sections 3 x 10 minutes in phosphate buffered saline.
8. Mount fluorescent sections in aqueous mountant or visualize peroxidase label (e.g. by exposure to freshly prepared 0.05% w/v diaminobenzidine in phosphate buffered saline containing 0.1% w/v hydrogen peroxide). Dehydrate, clear and mount peroxidase labelled sections for permanent preparations.

References

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