

Novocastra™ Lyophilized Mouse Monoclonal Antibody Utrophin (n-terminus)

Product Code: NCL-DRP2

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Amino terminal domain of the chromosome-6 encoded homolog of human dystrophin, utrophin, also known as dystrophin related protein or DRP). Also crossreacts with utrophin in sections of muscle from rat and dog. Other animal species have not been tested.
Clone	DRP3/20C5
Ig Class	IgG1
Antigen Used for Immunizations	Fusion protein containing the first 261 amino acids of the published DMDL gene sequence.
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 1:2–1:10. 60 minutes primary antibody incubation at 25 °C. Indirect immunoperoxidase technique (see overleaf). Electron microscopy gold: Light fixation with 2 per cent formaldehyde and 0.001 per cent 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. Western Blotting: Not recommended.
Positive Controls	Immunohistochemistry: Neuromuscular junctions (NMJs) can be located in the mid section of the rat soleus muscle using double labelling with fluorescent alpha-bungarotoxin to identify specific location. Western Blotting: Not recommended.
Staining Pattern	Light microscope: In normal adult human muscle the antibody labelling is restricted to NMJs, and does not label the general periphery of the muscle fibres. Blood vessels, capillaries and nerves are also labelled. Electron microscopy gold: At NMJs.
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	Utrophin, located on chromosome 6, is a ubiquitously expressed homolog of dystrophin and is known as dystrophin-related protein. In normal muscle, utrophin is restricted to neuromuscular junctions. However, in dystrophin-deficient muscle, utrophin expression may be upregulated and appears around the periphery of most fibres. Utrophin has a role as a cell anchoring molecule. The amino terminal region of utrophin binds to the actin cytoskeleton, acting as an intracellular anchor whereas the carboxyl terminal regions bind to a group of proteins anchored in the cell membrane.
General References	Winder S J, Gibson T J and Kendrick-Jones J. FEBS Letters. 369 (1): 27–33 (1995). Bewick G S, Nicholson L V B, Young C, et al.. NeuroReport. 3: 857–860 (1992).



Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).

Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).

Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).

Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

* 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).

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.