

Novocastra™ Lyophilized Mouse Monoclonal Antibody Gamma-Sarcoglycan

Product Code: NCL-g-SARC

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| Intended Use | FOR RESEARCH USE ONLY. |
| Specificity | Human gamma-sarcoglycan (35 kD). Also reacts strongly with gamma-sarcoglycan in mouse, dog and chicken, weakly with rat and rabbit gamma-sarcoglycan and very weakly with pig gamma-sarcoglycan. |
| Clone | 35DAG/21B5 |
| Ig Class | IgG2b, kappa |
| Antigen Used for Immunizations | Synthetic peptide containing amino acids 167–178 of the rabbit gamma-sarcoglycan sequence. (Science. 270: 819–822 (1995)). |
| 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). Electron microscopy gold: Light fixation with 2% formaldehyde + 0.001% glutaraldehyde for 1 hour, 2.3 M sucrose used as a cryoprotectant is recommended. Typical working dilution NEAT. 90 minutes primary antibody incubation at 25 °C. Western Blotting: Not recommended. |
| Positive Controls | Immunohistochemistry: Normal human striated muscle frozen in isopentane chilled in liquid nitrogen. Western Blotting: Normal human striated muscle. |
| Staining Pattern | Light microscope: continuous labelling around normal muscle fibres. Electron microscopy gold: continuous labelling around normal 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 | In normal skeletal muscle, dystrophin, the protein product of the gene which is defective in Duchenne and Becker dystrophy, is attached to the muscle membrane via a complex of at least seven proteins (dystrophin associated glycoproteins, DAGs) including the four sarcoglycans, alpha, beta, gamma and delta. Dystrophin-deficient muscle shows a generalised reduction in DAG labelling. Important: For reliable interpretation of 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). Noguchi S, McNally E M, Othmane K B, et al.. Science. 270: 819–822 (1995). |



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).