Product datasheet

Anti-Alpha Dystroglycan antibody [45-3] ab199768

Overview

Product name
Anti-Alpha Dystroglycan antibody [45-3]

Description
Rabbit monoclonal [45-3] to Alpha Dystroglycan

Host species
Rabbit

Tested applications
Suitable for: WB, IHC-P, ICC/IF

Species reactivity
Reacts with: Rat, Human

Immunogen
Recombinant fragment corresponding to Mouse Alpha Dystroglycan aa 450-700 (C terminal).
Database link: Q62165

General notes
This antibody clone is manufactured by Abcam.
If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form
Liquid

Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.40
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine

Purity
Protein G purified

Clonality
Monoclonal

Clone number
45-3

Applications

Our Abpromise guarantee covers the use of ab199768 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
The dystroglycan complex is involved in a number of processes including laminin and basement membrane assembly, sarcolemmal stability, cell survival, peripheral nerve myelination, nodal structure, cell migration, and epithelial polarization.

Alpha-dystroglycan is an extracellular peripheral glycoprotein that acts as a receptor for both extracellular matrix proteins containing laminin-G domains, and for certain adenoviruses. Receptor for laminin-2 (LAMA2) and agrin in peripheral nerve Schwann cells. Also acts as a receptor for M.leprae in peripheral nerve Schwann cells but only in the presence of the G-domain of LAMA2, and for lymphocytic choriomeningitis virus, Old World Lassa fever virus, and clade C New World arenaviruses.

Beta-dystroglycan is a transmembrane protein that plays important roles in connecting the extracellular matrix to the cytoskeleton. Acts as a cell adhesion receptor in both muscle and non-muscle tissues. Receptor for both DMD and UTRN and, through these interactions, scaffolds axin to the cytoskeleton. Also functions in cell adhesion-mediated signaling and implicated in cell polarity.

**Tissue specificity**
Expressed in a variety of fetal and adult tissues. In epidermal tissue, located to the basement membrane. Also expressed in keratinocytes and fibroblasts.

**Involvement in disease**
Defects in DAG1 are the cause of muscular dystrophy-dystroglycanopathy limb-girdle type C7 (MDDGC7) [MIM:613818]. An autosomal recessive muscular dystrophy showing onset in early childhood, and associated with mental retardation without structural brain anomalies.

**Sequence similarities**
Contains 1 peptidase S72 domain.

**Post-translational modifications**
O- and N-glycosylated. Alpha-dystroglycan is heavily O-glycosylated comprising of up to two thirds of its mass and the carbohydrate composition differs depending on tissue type. Mucin-type O-glycosylation is important for ligand binding activity. O-mannosylation of alpha-DAG1 is found in high abundance in both brain and muscle where the most abundant glycan is Sia-alpha-2-3-Gal-beta-1-4-Glc-NAc-beta-1-2-Man. In muscle, glycosylation on Thr-379 by a phosphorylated O-mannosyl glycan with the structure 2-(N-acetylamido)-2-deoxygalactosyl-beta-1,3-2-(N-acetylamido)-2-deoxyglucosyl-beta-1,4-6-phosphomannose is mediated by like-acetylglucosaminyltransferase (LARGE) protein and is required for laminin binding. O-mannosylation is also required for binding lymphocytic choriomeningitis virus, Old World Lassa fever virus, and clade C New World arenaviruses. The O-glycosyl hexose on Thr-367, Thr-369, Thr-372, Thr-381 and Thr-388 is probably mannose. O-glycosylated in the N-terminal region with a core 1 or possibly core 8 glycan. The beta subunit is N-glycosylated.

**Application**

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<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 95 kDa (predicted molecular weight: 97 kDa).</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
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</tbody>
</table>

**Target**

### Function
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Note=MDDGC7 is caused by DAG1 mutations that interfere with normal post-translational processing, resulting in defective DAG1 glycosylation and impaired interactions with extracellular-matrix components. Other muscular dystrophy-dystroglycanopathies are caused by defects in enzymes involved in protein O-glycosylation.

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Autolytic cleavage produces the alpha and beta subunits. In cutaneous cells, as well as in certain pathological conditions, shedding of beta-dystroglycan can occur releasing a peptide of about 30 kDa.

SRC-mediated phosphorylation of the PPXY motif of the beta subunit recruits SH2 domain-containing proteins, but inhibits binding to WWW domain-containing proteins, DMD and UTRN. This phosphorylation also inhibits nuclear entry.

**Cellular localization**

Secreted > extracellular space and Cell membrane. Cytoplasm > cytoskeleton. Nucleus > nucleoplasm. The monomeric form translocates to the nucleus via the action of importins and depends on RAN. Nuclear transport is inhibited by Tyr-892 phosphorylation. In skeletal muscle, this phosphorylated form locates to a vesicular internal membrane compartment. In peripheral nerves, localizes to the Schwann cell membrane. Colocalizes with ERM proteins in Schwann-cell microvilli.

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**Images**

Western blot - Anti-Alpha Dystroglycan antibody [45-3] (ab199768)

**All lanes**: Anti-Alpha Dystroglycan [45-3] (ab199768) at 1 µg/ml

**Lane 1**: Human placenta tissue lysate - total protein (ab29745)

**Lane 2**: Mouse placenta normal tissue lysate - total protein (ab29139)

**Lane 3**: Placenta (Rat) Whole Cell Lysate - normal tissue (ab29143)

**Lane 4**: Human skeletal muscle tissue lysate - total protein (ab29330)

**Lane 5**: Human lung normal tissue lysate - total protein (40 - 65 years) (ab30281)

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) at 50000 µg/ml

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 97 kDa

**Observed band size**: 95 kDa

*why is the actual band size different from the predicted?*

**Additional bands at**: 40 kDa, 55 kDa. We are unsure as to the identity of these extra bands.

**Exposure time**: 20 minutes
Immunocytochemistry/ Immunofluorescence - Anti-
Alpha Dystroglycan antibody [45-3] (ab199768)

ab199768 stained A431 cells. The cells were 100% methanol fixed for 5 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab199768 at 5µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) used at a 1/1000 dilution for 1 hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1 hour at room temperature.

Immunohistochemistry (Formalin/PFA-fixed paraffin-
embedded sections) - Anti-Alpha Dystroglycan antibody [45-3] (ab199768)

IHC image of Alpha Dystroglycan staining in Human normal placenta formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab199768, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

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