

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Dystroglycan in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Dystroglycan Gln28-Val749 Accession # Q14118
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>	<p>Detection of Human Dystroglycan by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line, SH-SY5Y human neuroblastoma cell line, human muscle tissue, and human placenta tissue. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Dystroglycan Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6868) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for α-Dystroglycan at approximately 100-160 kDa (as indicated) and β-Dystroglycan at approximately 42-44 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunohistochemistry</p> <p>Dystroglycan in Human Skeletal Muscle. Dystroglycan was detected in immersion fixed paraffin-embedded sections of human skeletal muscle using Sheep Anti-Human Dystroglycan Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6868) at 3 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to basement membrane. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Dystroglycan, also DAG-1 (Dystrophin-associated glycoprotein 1) is a 180-200 kDa heterodimeric adhesion molecule that links the cell cytoskeleton to the extracellular matrix. It is found on skeletal muscle, cardiac muscle, fibroblasts, smooth muscle and keratinocytes. DAG-1 binds multiple matrix molecules, including laminin-1 and -2, agrin, and perlecan. Intracellularly, the cytoplasmic tail of DAG-1 contributes to a large 400 kDa complex that interacts with the cytoskeleton. The human DAG-1 precursor is a type I transmembrane protein 895 amino acids (aa) in length. It contains a 27 aa signal sequence plus an 868 aa proform that undergoes autocatalysis to generate a 626 aa α-chain (aa 28-653), and a 242 aa β-chain. Mature DAG-1 is a heterodimer composed of noncovalently linked α- and β-chains. The α-chain possesses one potential Ig-like domain (aa 64-162), a mucin-like region (aa 316-485), and a peptidase S72 domain (aa 500-733). It is O-glycosylated and runs from 100-160 kDa in SDS-PAGE. The β-chain is N-glycosylated and runs at 42-44 kDa in SDS-Page. It possesses a short 95 aa extracellular region (aa 654-749) plus a 120 aa cytoplasmic domain (aa 776-895). Membrane cleavage of the β-chain causes dissociation of the heterodimer and generates a 30 kDa truncated form. Over aa 28-749, human DAG-1 shares 93% aa identity with mouse DAG-1.