Human Dystroglycan Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6868

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.

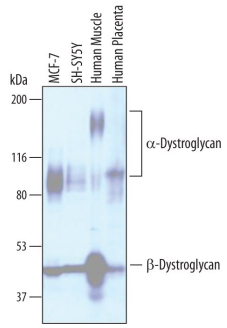
APPLICATIONS

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
Knockout Validated	Dystroglycan is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in Dystroglycan knockout HEK293T cell line.	

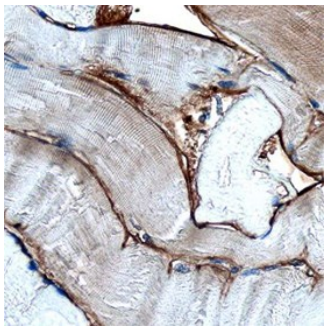
DATA

Western Blot



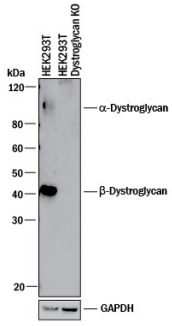
Detection of Human Dystroglycan by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line, SH-SY5Y human neuroblastoma cell line, human muscle tissue, and human placenta tissue. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Dystroglycan Antigen Affinity-purified Polyclonal 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.

Immunohistochemistry



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.

Knockout Validated



Western Blot Shows Human Dystroglycan Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and Dystroglycan knockout HEK293T cell line (KO). 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 110 kDa and β-Dystroglycan at approximately 42 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Human Dystroglycan Antibody

Antigen Affinity-purified Polyclonal Sheep IgG

Catalog Number: AF6868

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.** • 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 preprocursor 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.

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USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
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