Human Fascin Antibody

Monoclonal Mouse IgG_{2A} Clone # 833223

Catalog Number: MAB7745

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Fascin in ELISAs and Western blots. Detects mouse and rat Fascin in Western blots. In direct ELISAs, no cross-reactivity with recombinant human Fascin-2 is observed.
Source	Monoclonal Mouse IgG_{2A} Clone # 833223
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human Fascin Met1-Tyr493 Accession # Q16658
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.

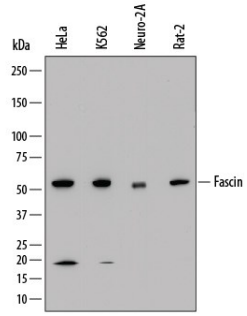
APPLICATIONS

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	8-25 µg/mL	See Below
Simple Western	10 µg/mL	See Below
Knockout Validated	Fascin is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in Fascin knockout HeLa cell line.	

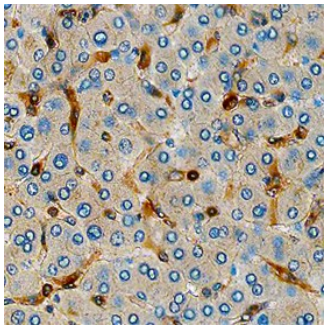
DATA

Western Blot



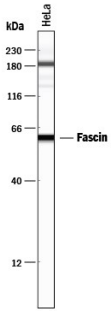
Detection of Human, Mouse, and Rat Fascin by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, K562 human chronic myelogenous leukemia cell line, Neuro-2A mouse neuroblastoma cell line, and Rat-2 rat embryonic fibroblast cell line. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Fascin Monoclonal Antibody (Catalog # MAB7745) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Fascin at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



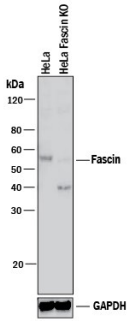
Fascin in Human Liver. Fascin was detected in immersion fixed paraffin-embedded sections of human liver using Mouse Anti-Human Fascin Monoclonal Antibody (Catalog # MAB7745) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to synusoids. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human Fascin by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line, loaded at 0.5 mg/mL. A specific band was detected for Fascin at approximately 61 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human Fascin Monoclonal Antibody (Catalog # MAB7745). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



Western Blot Shows Human Fascin Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and Fascin knockout HeLa cell line (KO). PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Fascin Monoclonal Antibody (Catalog # MAB7745) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Fascin at approximately 55 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

New adjunct appears with knockout cell line.

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Catalog Number: MAB7745

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 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

Fascin (*that which creates fascicles* [bundles] *of actin*; also known as 55 kDa actin-bundling protein, p55 and singed-like protein) is an intracellular 55-58 kDa member of the fascin family of proteins. It has a restricted expression pattern, being found in oligodendrocytes, select endothelium, cerebellar stellate neurons and blood, interdigitating, and thymic medullary dendritic cells. Fascin is found associated with actin in filopodia, and serves to coordinate and stabilize actin bundle formation, both in normal cells and tumor cells. In the latter cell type, filopodia have been renamed invadopodia, and their appearance is crucial for the creation of a stable platform that coordinates local matrix degradation. Human fascin is 493 amino acids (aa) in length. It contains an N-terminal fascin-like domain (aa 139-256) that contains part of one of two actin-binding sequences (aa 136-143), followed by two additional fascin-like domains (aa 260-378 and 383-493), the latter of which contains the second actin-binding sequence (aa 386-395). There are also two acetylation sites and two utilized phosphorylation sites at Ser38 and Ser39. Phosphorylation of the latter site inhibits fascin interaction with actin. At least two isoform variants may exist. One contains a 12 aa substitution for aa 427-493, while a second shows a deletion of aa 371-426. Full-length human fascin shares 97% aa sequence identity with mouse fascin. Two additional human fascins termed retinal and testis fascin have been identified. They are products of distinct genes and share 56% and 27% aa sequence identity with the standard (p55) fascin, respectively.

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