

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Endophilin B1/Bif-1 in direct ELISAs. In direct ELISAs, 100% cross-reactivity with recombinant human Endophilin B2 is observed.
Source	Monoclonal Mouse IgG _{2B} Clone # 807009
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Endophilin B1/Bif-1 Ala33-Asn189 Accession # Q9Y371
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 as a 0.2 µm filtered solution in PBS.

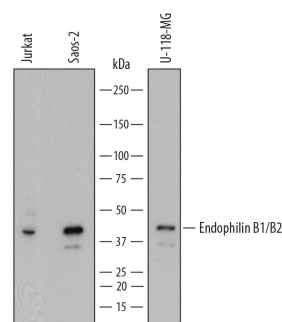
APPLICATIONS

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	0.2 µg/mL	See Below
Immunocytochemistry	5-25 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Simple Western	2 µg/mL	See Below

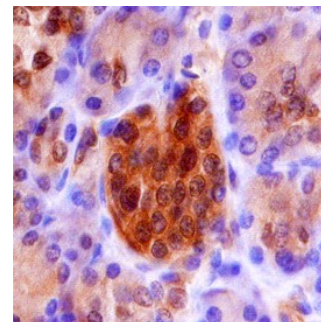
DATA

Western Blot



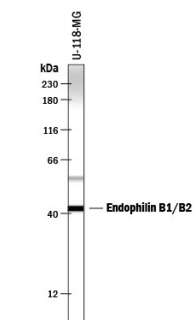
Detection of Human Endophilin B1/B2 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, Saos-2 human osteosarcoma cell line, and U-118-MG human glioblastoma/astrocytoma cell line. PVDF membrane was probed with 0.2 µg/mL of Mouse Anti-Human Endophilin B1/B2 Monoclonal Antibody (Catalog # MAB7456) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Endophilin B1/B2 at approximately 43 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

Immunohistochemistry



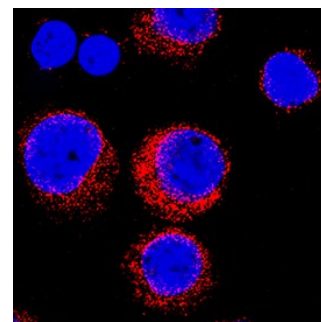
Endophilin B1/B2 in Human Pancreas. Endophilin B1/B2 was detected in immersion fixed paraffin-embedded sections of human pancreas using Mouse Anti-Human Endophilin B1/B2 Monoclonal Antibody (Catalog # MAB7456) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected 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 cytoplasm of islet cells. View our protocol for *Chromogenic IHC Staining of Paraffin-embedded Tissue Sections*.

Simple Western



Detection of Human Endophilin B1/B2 by Simple Western™. Simple Western lane view shows lysates of U-118-MG human glioblastoma/astrocytoma cell line, loaded at 0.5 mg/mL. A specific band was detected for Endophilin B1/B2 at approximately 42 kDa (as indicated) using 2 µg/mL of Mouse Anti-Human Endophilin B1/B2 Monoclonal Antibody (Catalog # MAB7456). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Immunocytochemistry



Endophilin B1/B2 in Jurkat Human Cell Line. Endophilin B1/B2 was detected in immersion fixed Jurkat human acute T cell leukemia cell line using Mouse Anti-Human Endophilin B1/B2 Monoclonal Antibody (Catalog # MAB7456) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for *Fluorescent ICC Staining of Non-adherent Cells*.

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. <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

Endophilins are among the best known BAR (Bin/Amphiphysin/Rvs) domain proteins. The highly conserved protein dimerisation domain is typically involved in membrane dynamics. Endophilin-B1, also known as SH3GLB1 (Src-Homology 3 Domain-containing GRB2-like protein B1) and BIF-1, is a 40 kDa protein that belongs to the endophilin family of molecules. It is a cytoplasmic and Golgi membrane protein that is expressed in neurons, striated (skeletal and cardiac) muscle cells, and placenta. Using SH3GLB1 in yeast two-hybrid screens, a second protein, SH3GLB2 (endophilin B2), was identified as an interacting partner. Endophilin-B1 plays a role in cell homeostasis. In neurons, Endophilin-B1 is phosphorylated by Cdk5, inducing homodimerization and autophagosome formation. In addition, it appears to play a role in the maintenance of mitochondrial integrity. Endophilin-B1 appears to cycle on-and-off the outer mitochondrial membrane (OMM), contributing to OMM integrity. And after the initiation of endocytosis, it also directs EEA1+ TrkA-containing endosomes back into the cell membrane for reuse. Human Endophilin-B1 is 365 amino acids (aa) in length. It possesses an N-terminal lipid-binding BAR (Bin/Amphiphysin/Rvs) domain (aa 27-261) that contains an internal coiled-coil region, and a C-terminal SH3 domain that binds Pro-rich sequences on potential ligands (309-363). There are at least three potential splice variants for Endophilin-B1. Two are 42 and 43 kDa in size, and possess a 21 and 29 aa insertion after Ser190, respectively. A third potentially utilizes an alternative start site at Met101. Over aa 33-189, human Endophilin-B1 shares 98% aa sequence identity with mouse Endophilin-B1.