Human/Mouse/Rat GIT1 Antibody

Monoclonal Mouse IgG_{2B} Clone # 924640

Catalog Number: MAB8508

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human GIT1 in direct ELISAs and human, mouse, and rat GIT1 in Western blots. In sandwich immunoassays, this antibody is specific for human GIT1 when paired with the suggested detection antibody.
Source	Monoclonal Mouse IgG_{2B} Clone # 924640
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human GIT1 Ser485-Asp636 Accession # Q9Y2X7
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.

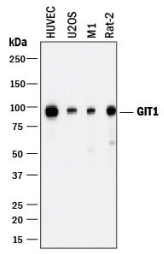
APPLICATIONS

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	2 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
ELISA	This antibody functions as an ELISA capture antibody when paired with Mouse Anti-Human GIT1 Monoclonal Antibody (Catalog # MAB85081). In sandwich immunoassays, this antibody is specific for human GIT1 when paired with the suggested detection antibody. *This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human GIT1 DuoSet ELISA Kit (Catalog # DY8485-05) for convenient development of a sandwich ELISA.*	

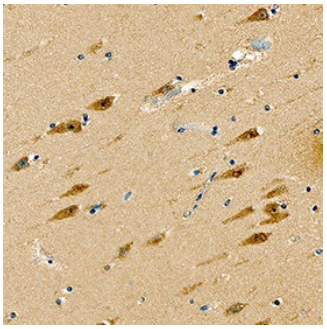
DATA

Western Blot



Detection of Human, Mouse, and Rat GIT1 by Western Blot. Western blot shows lysates of HUVEC human umbilical vein endothelial cells, U2OS human osteosarcoma cell line, M1 mouse myeloid leukemia cell line, and Rat-2 rat embryonic fibroblast cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human GIT1 Monoclonal Antibody (Catalog # MAB8508) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for GIT1 at approximately 95 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



GIT1 in Human Brain. GIT1 was detected in immersion fixed paraffin-embedded sections of human brain (hippocampus) using Mouse Anti-Human GIT1 Monoclonal Antibody (Catalog # MAB8508) at 15 µg/mL overnight at 4 °C. 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 neurons. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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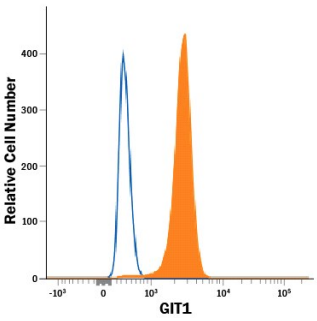
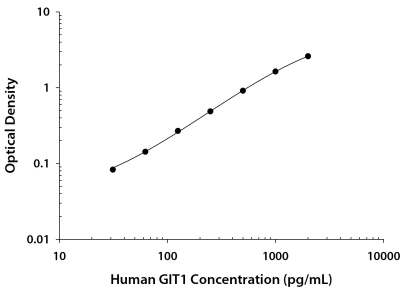
Intracellular Staining by Flow Cytometry



Detection of GIT1 in SH-SY5Y Human Cell line by Flow Cytometry. SH-SY5Y human neuroblastoma cell line was stained with Mouse Anti-Human GIT1 Monoclonal Antibody (Catalog # MAB8508, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).

ELISA



Human GIT1 ELISA Standard Curve. Recombinant Human GIT1 protein was serially diluted 2-fold and captured by Mouse Anti-Human/Mouse/Rat GIT1 Monoclonal Antibody (Catalog # MAB8508) coated on a Clear Polystyrene Microplate (Catalog # DY990). Mouse Anti-Human GIT1 Monoclonal Antibody (Catalog # MAB85081) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # DY998) followed by Substrate Solution (Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # DY994).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
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

GIT1 (ARF GTPase-activating protein GIT1) is a 95 kDa protein that belongs to ADP ribosylation factor family and is localized to focal adhesions, cytoplasmic complexes and membrane protrusions, and regulates cell protrusion formation and cell migration. G-protein coupled receptor (GPCR) kinase interacting proteins 1 and 2 (GIT1 and GIT2) are highly conserved, ubiquitous scaffold proteins involved in localized signaling to help regulate focal contact assembly and cytoskeletal dynamics. GIT proteins contain multiple interaction domains that allow interaction with small GTPases (including ARF, Rac and cdc42), kinases (such as PAK and MEK), the Rho family GEF PIX, and the focal adhesion protein paxillin. GIT1 has also been implicated in neuronal functions including synapse formation and the pathology of Huntington disease. Huntington disease is a genetic neurodegenerative condition involving a mutation in the huntington gene. The huntington gene product (htt) is ubiquitinated and degraded in human Huntington disease brains. Htt interacts directly with GIT1 causing enhanced htt proteolysis, indicating that GIT1 distribution and function may contribute to Huntington disease pathology. Within amino acids (aa) 485-636, human and mouse GIT1 share 93% aa sequence identity.

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