Human/Mouse/Rat STAT3 Antibody

Monoclonal Mouse IgG_{2B} Clone # 232209

Catalog Number: MAB1799

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat STAT3 in Western blots.
Source	Monoclonal Mouse IgG_{2B} Clone # 232209
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human STAT3 Met1-Asn175 Accession # P40763
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.

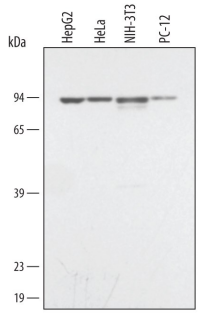
APPLICATIONS

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	0.1 µg/mL	See Below
Immunocytochemistry	3-25 µg/mL	See Below
Immunoprecipitation	1-3 µg/500 µg cell lysate	Daudi human Burkitt's lymphoma cell line, see our available Western blot detection antibodies
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Knockout Validated	STAT3 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in STAT3 knockout HeLa cell line.	

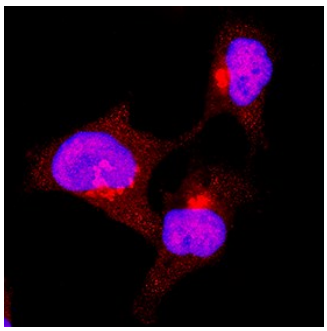
DATA

Western Blot



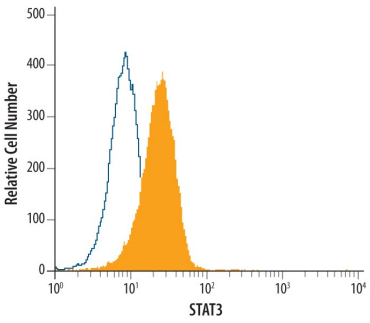
Detection of Human STAT3 by Western Blot. Western blot shows lysates of HepG2 human hepatocellular carcinoma cell line, HeLa human cervical epithelial carcinoma cell line, NIH-3T3 mouse embryonic fibroblast cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF membrane was probed with 0.1 µg/mL of Mouse Anti-Human STAT3 Monoclonal Antibody (Catalog # MAB1799) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF007). A specific band was detected for STAT3 at approximately 90 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



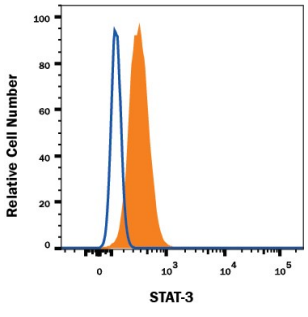
STAT3 in HeLa Human Cell Line. STAT3 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Mouse Anti-Human/Mouse/Rat STAT3 Monoclonal Antibody (Catalog # MAB1799) at 3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of STAT3 in Jurkat Human Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line was stained with Mouse Anti-Human STAT3 Monoclonal Antibody (Catalog # MAB1799, filled histogram) or isotype control antibody (Catalog # Catalog # MAB0041, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG $F(ab')_2$Secondary Antibody (Catalog # Catalog # F0101B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with methanol.

Intracellular Staining by Flow Cytometry



Detection of STAT3 in HeLa Human Cell Line by Flow Cytometry. HeLa human cell line was stained with Mouse Anti-Human/Mouse/Rat STAT3 Monoclonal Antibody (Catalog # MAB1799, filled histogram) or isotype control antibody (Catalog # Catalog # MAB0041, open histogram) followed by anti-Mouse IgG PE-conjugated secondary antibody (Catalog # Catalog # F0102B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # Catalog # FC005). View our protocol for Staining Intracellular Molecules.

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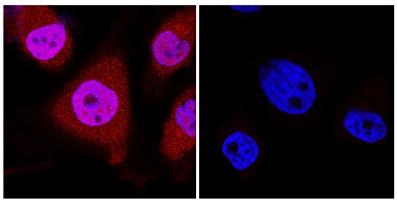
Human/Mouse/Rat STAT3 Antibody

Monoclonal Mouse IgG_{2B} Clone # 232209

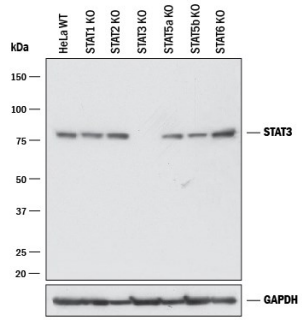
Catalog Number: MAB1799

Knockout Validated



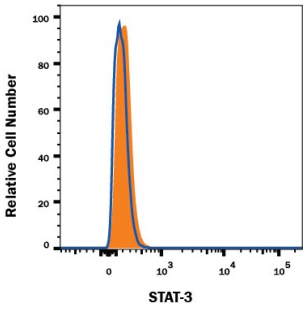
STAT3 Specificity is Shown by Immunocytochemistry in Knockout Cell Line. STAT3 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with IFN-alpha 1, but is not detected in STAT3 knockout (KO) HeLa cell line using Mouse Anti-Human/Mouse/Rat STAT3 Monoclonal Antibody (Catalog # MAB1799) at 1 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Knockout Validated



Western Blot Shows Human STAT3 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line, STAT1 knockout (KO) HeLa cell line, STAT2 KO HeLa cell line, STAT3 KO HeLa cell line, STAT5a KO HeLa cell line, STAT5b KO HeLa cell line, STAT6 KO HeLa cell line,. PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human/Mouse/Rat STAT3 Monoclonal Antibody (Catalog # MAB1799) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF018). A specific band was detected for STAT3 at approximately 80 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the STAT3 knockout HeLa cell line. GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Knockout Validated



STAT3 Specificity is Shown by Flow Cytometry in Knockout Cell Line. STAT3 knockout HeLa human cervical epithelial cell line was stained with Mouse Anti-Human/Mouse STAT3 Monoclonal Antibody (Catalog # MAB1799, filled histogram) or isotype control antibody (Catalog # Catalog # MAB0041, open histogram) followed by anti-Mouse IgG PE-conjugated secondary antibody (Catalog # Catalog # F0102B). No staining in the STAT3 knockout HeLa cell line was observed. To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # Catalog # FC005). View our protocol for Staining Intracellular Molecules.

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

Signal Transducer and Activator of Transcription (STAT) proteins are transcription factors activated in response to cytokine, growth factor, or hormone receptor signaling. Janus kinases (JAKs) phosphorylate STAT proteins and induce dimerization. Homo- or heterodimers translocate to the nucleus where they bind to DNA and activate transcription.

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