

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human STAT5a/b when phosphorylated at Y964/Y699 in direct ELISAs and Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1247C
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Phosphopeptide containing human STAT5b Y699 site (amino acid sequence of this peptide is identical to a corresponding region of human STAT5a containing Y694) Accession # P51692
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
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.	

DATA

<p>Western Blot</p> <p>Detection of Human Phospho-STAT5a/b (Y694/Y699) by Western Blot. Western blot shows lysates of Daudi human Burkitt's lymphoma cell line and HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 500 U/mL Recombinant Human IFN-αA (Catalog # 11100-1) for 20 minutes. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human Phospho-STAT5a/b (Y694/Y699) Monoclonal Antibody (Catalog # MAB41901) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for STAT5a/b at approximately 95 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Intracellular Staining by Flow Cytometry</p> <p>Detection of Phospho-STAT5a/b (Y694/Y699) in IFN alpha-treated Daudi Human Cell Line by Flow Cytometry. Daudi human Burkitt's lymphoma cell line was unstimulated (open histogram) or treated with 500 U/mL rhIFN-alpha for 20 minutes (filled histogram) and stained with Rabbit anti-Human Phospho-STAT5a/b (Y694/Y699) Monoclonal Antibody (Catalog # MAB41901) followed by APC-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with methanol. View our protocol for Staining Intracellular Molecules.</p>
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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. <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

Signal transduction and activator of transcription 5 (STAT5) is a member of the Jak/STAT signal transduction pathway and is activated by a variety of cytokines (IL-22, IL-6, IFN-α). STAT5 has two isoforms (A and B) that share 93% amino acid identity and bind the DNA consensus site TTCN₃GAA. STAT5 mediates cytokine signaling by acting as a signal transducer in the cytoplasm and, upon phosphorylation, translocates to the nucleus and activates transcription of specific genes. STAT5 is involved in a wide array of biological processes ranging from regulating apoptosis to adult mammary gland proliferation, differentiation, and survival.