

DESCRIPTION

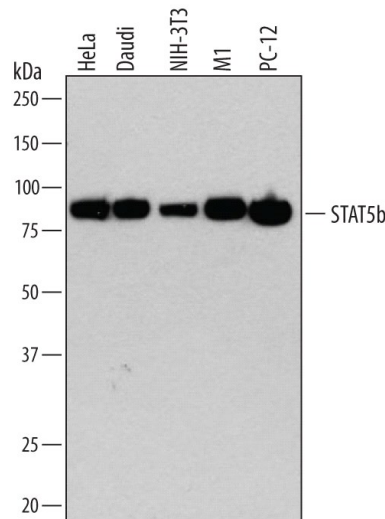
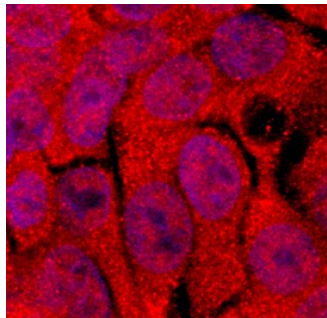
Species Reactivity	Human/Mouse
Specificity	Detects human and mouse STAT5b.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Human STAT5b synthetic peptide aa 777-787
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

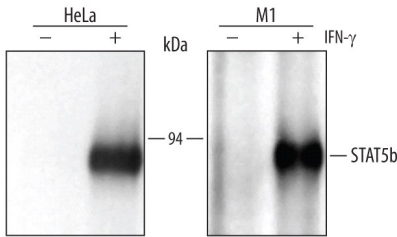
Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.2 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunoprecipitation	10 µg/10 ⁶ cells	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10 ⁶ cells	See Below
Simple Western	10 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

DATA

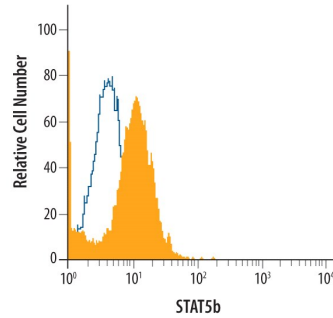
<p>Western Blot</p>  <p>Detection of Human, Mouse, and Rat STAT5b by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, Daudi human Burkitt's lymphoma cell line, NIH-3T3 mouse embryonic fibroblast cell line, M1 mouse myeloid leukemia cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF membrane was probed with 0.2 µg/mL of Rabbit Anti-Human/Mouse STAT5b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1584) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for STAT5b at approximately 90 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>STAT5b in HepG2 Human Cell Line. STAT5b was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit Anti-Human/Mouse STAT5b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1584) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and nucleus. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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Immunoprecipitation



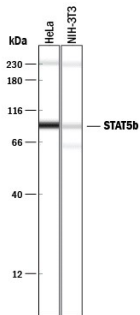
Immunoprecipitation of Human and Mouse STAT5b. HeLa human cervical epithelial carcinoma and M1 mouse myeloid leukemia cell line were untreated (-) or activated (+) with 100 ng/mL Recombinant Human IFN- γ (Catalog # 285-IF) for 15 minutes. STAT5b was immunoprecipitated from lysates of 5×10^6 cells following incubation with 2 μ g Rabbit Anti-Human/Mouse STAT5b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1584) for 1 hour at room temperature. STAT5b-antibody complexes were absorbed using Protein A sepharose (Invitrogen, Catalog # 10-1041). Immunoprecipitated STAT5b was detected by Western blot using 1 μ g/mL Human Phospho-STAT5a/b (Y699) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4190). View our [recommended buffer recipes for immunoprecipitation](#).

Intracellular Staining by Flow Cytometry



Detection of STAT5b in Jurkat Human Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line was stained with Rabbit Anti-Human/Mouse STAT5b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1584, filled histogram) or control antibody (Catalog # AB-105-C, open histogram), followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with methanol.

Simple Western



Detection of Human and Mouse STAT5b by Simple Western™.

Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and NIH-3T3 mouse embryonic fibroblast cell line, loaded at 0.2 mg/mL. A specific band was detected for STAT5b at approximately 92 kDa (as indicated) using 10 μ g/mL of Rabbit Anti-Human/Mouse STAT5b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1584). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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.