

## DESCRIPTION

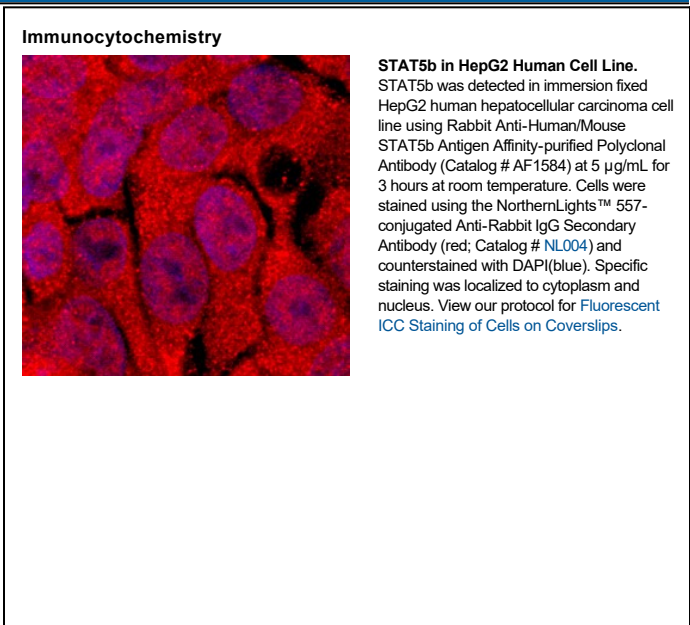
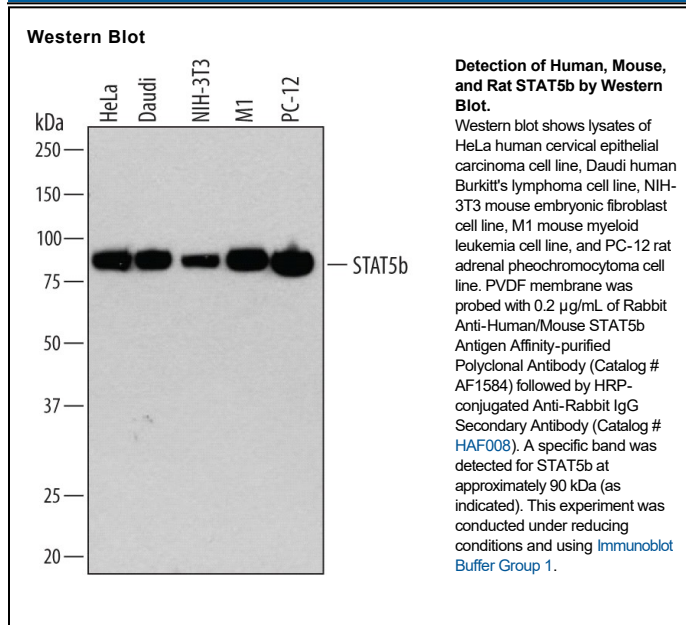
<b>Species Reactivity</b>	Human/Mouse
<b>Specificity</b>	Detects human and mouse STAT5b.
<b>Source</b>	Polyclonal Rabbit IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Human STAT5b synthetic peptide aa 777-787
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with 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

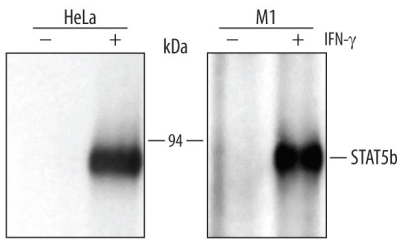
**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
<b>Western Blot</b>	0.2 µg/mL	See Below
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Immunoprecipitation</b>	10 µg/10 <sup>6</sup> cells	See Below
<b>Intracellular Staining by Flow Cytometry</b>	2.5 µg/10 <sup>6</sup> cells	See Below
<b>Simple Western</b>	10 µg/mL	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
<b>Knockout Validated</b>	STAT5b is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in STAT5b knockout HeLa cell line.	

## DATA

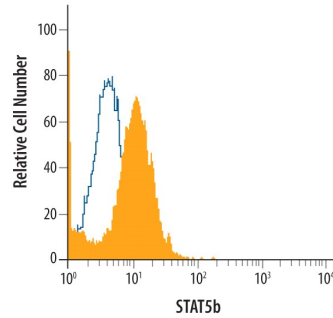


## Immunoprecipitation



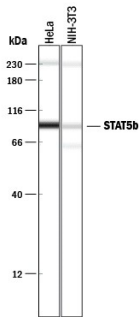
**Immunoprecipitation of Human and Mouse STAT5b.** HeLa human cervical epithelial carcinoma and M1 mouse myeloid leukemia cell line were untreated (-) or activated (+) with 100 ng/mL Recombinant Human IFN- $\gamma$  (Catalog # 285-IF) for 15 minutes. STAT5b was immunoprecipitated from lysates of  $5 \times 10^6$  cells following incubation with 2  $\mu$ 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  $\mu$ 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

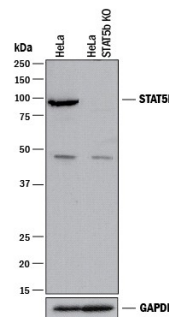


**Detection of Human and Mouse STAT5b by Simple Western™.** Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and NIH-3T3 mouse embryonic fibroblast cell line, loaded at 0.2 mg/mL. A specific band was detected for STAT5b at approximately 92 kDa (as indicated) using 10  $\mu$ 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.



## Knockout Validated



**Western Blot Shows Human STAT5b Specificity by Using Knockout Cell Line.** Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and STAT5b knockout HeLa cell line (KO). PVDF membrane was probed with 0.2  $\mu$ g/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) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>