

**DESCRIPTION**

<b>Species Reactivity</b>	Human/Mouse/Rat
<b>Specificity</b>	Detects human, mouse and rat Akt1, Akt2 and Akt3, when phosphorylated at S473, S474 and S472, respectively.
<b>Source</b>	Polyclonal Rabbit IgG
<b>Purification</b>	Antigen and protein A Affinity-purified
<b>Immunogen</b>	Phosphopeptide containing human, mouse, and rat Akt1 S473 site
<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.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	0.5 µg/mL	See Below
<b>Immunohistochemistry</b>	5-15 µg/mL	See Below
<b>Intracellular Staining by Flow Cytometry</b>	2.5 µg/10 <sup>6</sup> cells	See Below
<b>Simple Western</b>	5 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.	

**DATA**

**Western Blot**

**Detection of Mouse and Human Phospho-Akt (S473) Pan Specific by Western Blot.** Western blot shows lysates of NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 100 ng/mL Human PDGF (Catalog # 120-HD) for 15 minutes and MCF-7 human breast cancer cell line untreated or treated with 100 ng/mL Recombinant Human IGF-1 (Catalog # 291-G1) for 15 minutes. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-Akt (S473) Pan Specific Antigen Affinity-purified Polyclonal Antibody (Catalog # AF887), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-Akt (S473) Pan Specific at approximately 60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Immunohistochemistry**

**Akt in Human Breast Cancer Tissue.** Akt phosphorylated at S473 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Rabbit Anti-Human/Mouse/Rat Phospho-Akt (S473) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF887) at 10 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

**Intracellular Staining by Flow Cytometry**

**Detection of Phospho-Akt (S473) in IGF-1-treated MCF-7 cells by Flow Cytometry.** Serum-starved MCF-7 human breast cancer cells were unstimulated (light orange filled histogram) or activated with 100 ng/mL Recombinant human IGF-1 (Catalog # 291-G1, dark orange filled histogram) for 15 minutes, then stained with Rabbit Anti-Human/Mouse/Rat Phospho-Akt (S473) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF887) 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 saponin.

**Simple Western**

**Detection of Human Phospho-Akt (S473) by Simple Western™.** Simple Western lane view shows lysates of MCF-7 human breast cancer cell line and A549 human lung carcinoma cell line untreated (-) or treated (+) with 100 ng/mL Recombinant Human IGF-1 (Catalog # 291-G1) for 15 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-Akt (S473) at approximately 60 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-Akt (S473) Pan Specific Antigen Affinity-purified Polyclonal Antibody (Catalog # AF887). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

**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>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <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>