

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse and rat Akt1, Akt2 and Akt3, when phosphorylated at S473, S474 and S472, respectively.
Source	Polyclonal Rabbit IgG
Purification	Antigen and protein A Affinity-purified
Immunogen	Phosphopeptide containing human, mouse, and rat Akt1 S473 site
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	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10 ⁶ cells	See Below
Simple Western	5 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

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

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 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.