Human/Mouse Phospho-Akt (S473) Pan Specific Antibody

Monoclonal Mouse IgG_1 Clone # 545007

Catalog Number: MAB887

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse Akt1, Akt2 and Akt3, when phosphorylated at S473, S474 and S472, respectively.
Source	Monoclonal Mouse IgG_1 Clone # 545007
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human Akt (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 either lyophilized or as a 0.2 µm filtered solution in PBS.

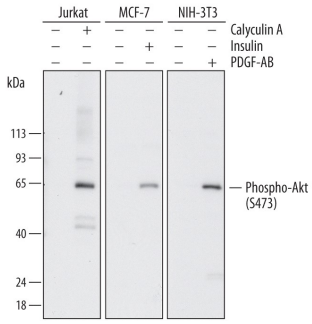
APPLICATIONS

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	0.2 µg/mL	See Below
Simple Western	2 µg/mL	See Below

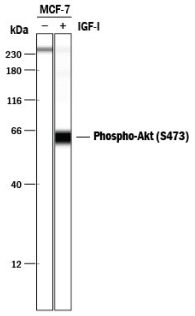
DATA

Western Blot



Detection of Human and Mouse Phospho-Akt (S473) by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, MCF-7 human breast cancer cell line, and NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 100 nm Calyculin for 30 minutes, 1 µg/mL insulin for 5 minutes, or 100 ng/mL Recombinant Human PDGF-AB (Catalog # 222-AB) for 20 minutes. PVDF membrane was probed with 0.2 µg/mL of Mouse Anti-Human Phospho-Akt (S473) Pan Specific Monoclonal Antibody (Catalog # MAB887) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Phospho-Akt (S473) at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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 untreated (-) or treated (+) with 100 ng/mL Recombinant Human IGF-I (Catalog # 291-G1) for 20 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-Akt (S473) at approximately 65 kDa (as indicated) using 2 µg/mL of Mouse Anti-Human/Mouse Phospho-Akt (S473) Pan Specific Monoclonal Antibody (Catalog # MAB887). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
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.

BACKGROUND

Akt, also known as protein kinase B (PKB), is a central kinase in such diverse cellular processes as glucose uptake, cell cycle progression, and apoptosis. Three highly homologous members define the Akt family: Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). All three Akts contain an amino-terminal pleckstrin homology domain, a central kinase domain, and a carboxyl-terminal regulatory domain. Akt1 is the most widely expressed family member and is frequently activated in a number of carcinomas, including breast, prostate, lung, pancreatic, liver, ovarian, and colorectal cancer. Akt1 is activated in a multistep process that involves the sequential phosphorylation of Thr450 by JNK kinases, Thr308 by PDK1, and Ser473 by PDK2 or mTORC2. Activated Akt1 phosphorylates a wide variety of cytosolic, nuclear, and mitochondrial substrates. Human Akt1 shares 98% aa sequence identity with mouse and rat Akt1.

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