

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse and rat Akt in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG _{2B} Clone # 281046
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Akt1 Ser2-Ala480 Accession # P31749
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.

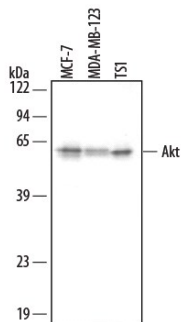
APPLICATIONS

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	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10 ⁶ cells	See Below
Simple Western	2 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.	

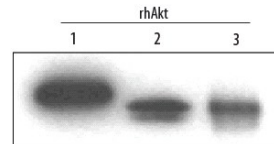
DATA

Western Blot



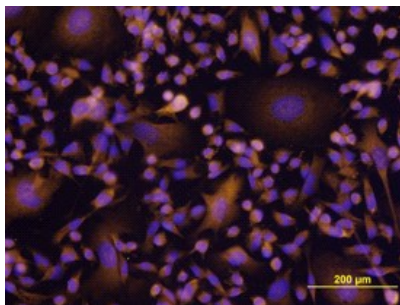
Detection of Human/Mouse Akt by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line, MDA-MB-123 human breast cancer cell line, and TS1 mouse helper T cell line. PVDF membrane was probed with 0.2 µg/mL Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # MAB2055) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

Western Blot



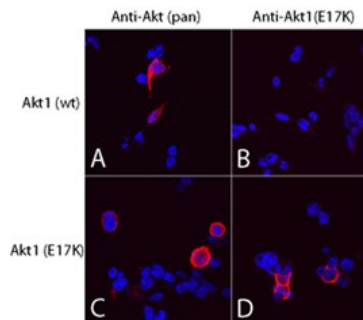
Detection of Human Akt Pan Specific by Western Blot. Western blot shows Recombinant Human Active Akt1 (Catalog # 1775-KS), recombinant human Akt2, and recombinant human Akt3 (5 ng/lane). PVDF membrane was probed with 0.2 µg/mL Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # MAB2055) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

Immunocytochemistry



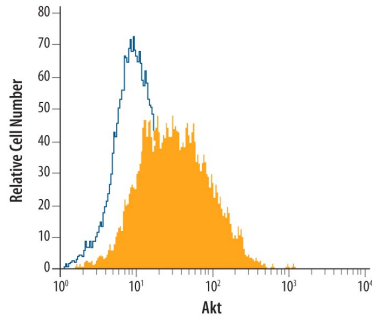
Akt in MDA-MB-231 Human Cell Line. Akt was detected in immersion fixed MDA-MB-231 human breast cancer cell line using Mouse Anti-Human/Mouse/Rat Akt Monoclonal Antibody (Catalog # MAB2055) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (yellow; Catalog # NL007) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



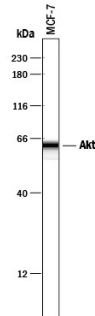
Akt (pan) in 293T Human Cell Line. Akt pan specific (panels A and C) and Akt1 (E17K Mutation) (panels B and D) were detected in immersion fixed 293T human embryonic kidney cell line transfected with wild type (panels A and B) or E17K mutated (panels C and D) Akt1 using Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # MAB2055) and Mouse Anti-Human Akt1 (E17K Mutation) Monoclonal Antibody (Catalog # MAB6815). Both antibodies were used at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to plasma membranes and cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Intracellular Staining by Flow Cytometry



Detection of Akt in MCF-7 Human Cell Line by Flow Cytometry. MCF-7 human breast cancer cell line was stained with Mouse Anti-Human/Mouse/Rat Akt Monoclonal Antibody (Catalog # MAB2055, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG F(ab')₂ Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

Simple Western



Detection of Human Akt by Simple Western™. Simple Western lane view shows lysates of MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for Akt at approximately 62 kDa (as indicated) using 2 µg/mL of Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # MAB2055). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution Reconstitute at 0.5 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.

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 (PKB α), Akt2 (PKB β), and Akt3 (PKB γ). All three Akts contain an amino-terminal pleckstrin homology domain, a central kinase domain, and a carboxyl-terminal regulatory domain.