

## DESCRIPTION

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

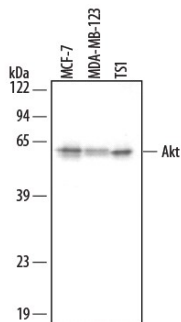
## APPLICATIONS

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>	8-25 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>	2 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.	

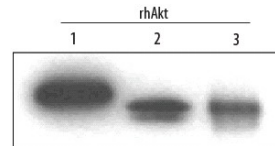
## DATA

### Western Blot



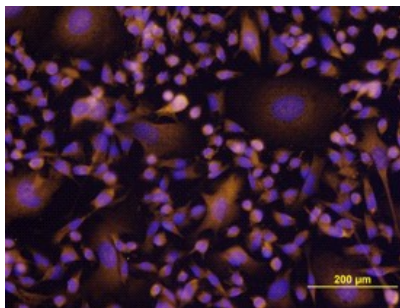
**Detection of Human/Mouse Akt by Western Blot.** Western blot shows lysates of MCF-7 human breast cancer cell line, MBA-MB-123 human breast cancer cell line, and TS1 mouse helper T cell line. PVDF membrane was probed with 0.2 µg/mL Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # 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



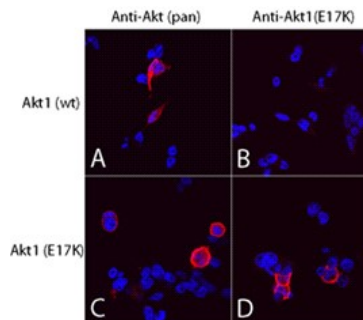
**Detection of Human Akt Pan Specific by Western Blot.** Western blot shows Recombinant Human Active Akt1 (Catalog # 1775-KS), recombinant human Akt2, and recombinant human Akt3 (5 ng/lane). PVDF membrane was probed with 0.2 µg/mL Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # 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



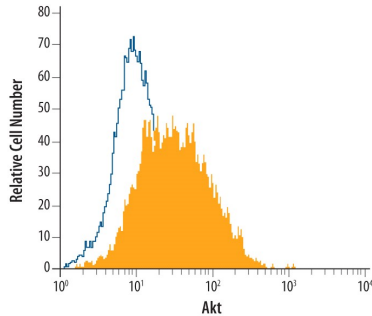
**Akt in MDA-MB-231 Human Cell Line.** Akt was detected in immersion fixed MDA-MB-231 human breast cancer cell line using Mouse Anti-Human/Mouse/Rat Akt Monoclonal Antibody (Catalog # MAB2055) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse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

### Immunocytochemistry



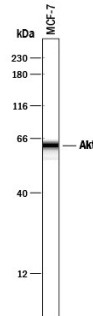
**Akt (pan) in 293T Human Cell Line.** Akt pan specific (panels A and C) and Akt1 (E17K Mutation) (panels B and D) were detected in immersion fixed 293T human embryonic kidney cell line transfected with wild type (panels A and B) or E17K mutated (panels C and D) Akt1 using Mouse Anti-Human/Mouse/Rat 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')<sub>2</sub> 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

<b>Reconstitution</b>	Reconstitute at 0.5 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>

### 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 $\alpha$ ), Akt2 (PKB $\beta$ ), and Akt3 (PKB $\gamma$ ). All three Akts contain an amino-terminal pleckstrin homology domain, a central kinase domain, and a carboxyl-terminal regulatory domain.