

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human AKT1 with Glu17Lys mutation in Western blots. In Western blots, no cross-reactivity with 293-EBNA human EBV-expressing embryonic kidney cell line transfectant expressing wild type AKT1 is detected.
Source	Monoclonal Mouse IgG ₁ Clone # 710022
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic peptide containing human Akt1 Ala5-Lys30 with Glu17Lys mutation 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.

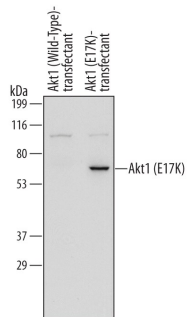
APPLICATIONS

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	0.5 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

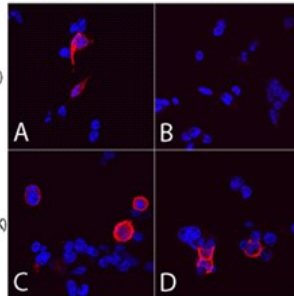
DATA

Western Blot



Detection of Human Akt1 (E17K Mutation) by Western Blot. Western blot shows lysates of 293-EBNA human EBV-expressing embryonic kidney cell line either transfected with Akt1 (wild-type) or transfected with Akt1 (E17K Mutation). PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human Akt1 (E17K Mutation) Monoclonal Antibody (Catalog # MAB6815) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Akt1 (E17K Mutation) at approximately 60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Akt1 (E17K Mutation) 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 Akt1 (panels A and B) or E17K mutated Akt1 (panels C and D) using Mouse Anti-Human Akt1 (E17K Mutation) Monoclonal Antibody (Catalog # MAB6815) and Mouse Anti-Human/Mouse/Rat Akt Pan Specific Monoclonal Antibody (Catalog # MAB2055). 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](#).

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. <ul style="list-style-type: none"> • 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. 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. Substitution of glutamic acid with lysine at amino acid 17 (E17K) occurs in multiple malignancies and results in constitutive Akt1 activation and cell transformation. Within aa 5-30, human Akt1 shares 100% aa sequence identity with mouse and rat Akt1.

References:

1. Carpten, J.D. *et al.* (2007) *Nature* **448**:439.
2. Bleeker, F.E. *et al.* (2008) *Oncogene* **27**:5648.
3. Brugge, J. *et al.* *Cancer Cell* **12**:104.