

Product Information

Anti-phospho-AKT (pThr⁴⁵⁰) antibody, Mouse monoclonal
clone AK-11, purified from hybridoma cell culture

Product Number **SAB4200753**

Product Description

Anti-phospho-AKT (pThr⁴⁵⁰) antibody, Mouse monoclonal (mouse IgG2a isotype) is derived from the AK-11 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized BALB/C mouse. Synthetic peptide corresponding to human AKT1 pThr450 (GenelD: 207) conjugated to KLH, was used as the immunogen. This sequence is identical in mouse, rat, monkey, bovine and chicken and is highly conserved in AKT2 and AKT3 (single amino acid substitution). The isotype is determined by ELISA using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2. The antibody is purified from culture supernatant of hybridoma cells.

Anti-phospho-AKT (pThr⁴⁵⁰) antibody, Mouse monoclonal specifically recognizes all three AKT isoforms phosphorylated at Thr⁴⁵⁰ (AKT1, AKT2 and AKT3). The antibody shows reactivity with human, mouse, rat and chicken AKT. The antibody may be used in various immunochemical techniques including Immunoblotting (~56kDa), Immunofluorescence and Immunoprecipitation. Staining of phospho-AKT (pThr⁴⁵⁰) by Immunoblotting is specifically inhibited with the phospho-Thr⁴⁵⁰-AKT immunizing peptide and is not inhibited with the corresponding non-phosphorylated peptide.

AKT also known as Protein kinase B (PKB) RAC-serine/threonine-protein kinase or Proto-oncogene c-Akt, belongs to a family of serine/threonine kinases considered to play an important role in the control of cell cycle, cell proliferation, differentiation and apoptosis signaling pathways.¹⁻² Three isoforms of AKT have been identified and characterized: AKT1, (PKB α), Akt2 (PKB β) and AKT3 (PKB γ).⁴⁻⁵ AKTs are rapidly activated in response to cell stimulation by several growth factors, insulin, peroxyvanadate or cellular stresses (such as heat shock). Following activation the phosphorylated AKT is relocated to the nucleus.¹⁻⁵ AKT is activated through a series of phosphorylation steps; first, AKT is phosphorylated at Thr⁴⁵⁰ by JNK kinases to prime its activation; second, phosphoinositide-dependent kinase 1 (PDK1) phosphorylates AKT at Thr³⁰⁸ to expose its Ser⁴⁷³ residue; third, AKT is phosphorylated at Ser⁴⁷³ by several kinases (including PKD2 and other kinases).³⁻⁵ AKT signaling is terminated by a series of

dephosphorylation steps initiated by the lipid phosphatase PTEN, through dephosphorylation of PIP3. Serine/threonine phosphatase-1 (PP-1) dephosphorylates AKT at Thr⁴⁵⁰.^{3,6-8} Inhibition of phosphorylation on Thr⁴⁵⁰ results in reduced AKT signaling pathway activity,⁶ while lack of phosphorylation at Thr⁴⁵⁰ enhances AKT ubiquitination and degradation.⁷⁻⁸

AKT1 plays a crucial role in different cell types and was demonstrated as a suppressor of apoptotic cell death induced by a variety of stimuli including growth factor withdrawal, loss of cell adhesion, and DNA damage.^{1,9-11}

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody Concentration: ~ 1.0 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2–8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 1–2 μ g/mL is recommended using human breast cancer cell line MCF-7 extract.

Immunofluorescence: a working concentration of 1–2 μ g/mL is recommended using mouse embryo fibroblast NIH-3T3 cells.

Immunoprecipitation: a working amount of 2–4 µg/test is recommended using whole extract of mouse embryo fibroblast NIH-3T3 cell line.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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