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ProductInformation

ANTI-phospho-PKB (pSer^{473})
Developed in Rabbit, IgG Fraction of Antiserum

Product Number **P 4112**

Product Description

Anti-phospho-PKB (pSer^{473}) is developed in rabbit using a synthetic phosphorylated peptide corresponding to (pSer^{473}) PKBα (human, amino acids 461-477 with N-terminally added lysine), conjugated to KLH as immunogen. This sequence is identical in mouse, rat and bovine PKBα and viral Akt. It is highly conserved in chicken PKBα (single amino acid substitution) and to a lesser extent in PKBβ (64%) and PKBγ (88%). Whole antiserum is fractionated and purified by ion-exchange chromatography. The resulting IgG fraction is further purified by specific absorption on the corresponding non-phosphorylated PKBα peptide (human, amino acids 462-477 with N-terminally added lysine), to remove undesired antibodies to non-phosphorylated PKB, and thus to obtain the specific phospho-PKB (pSer^{473}) antibody.

Anti-phospho-PKB (pSer^{473}) recognizes PKB phosphorylated at pSer^{473} (56 kDa). The antibody may be used for detection and localization of phospho-PKB by immunoblotting. Staining of phospho-PKB by immunoblotting is specifically inhibited with the phosphoSer^{473}-PKB immunizing peptide.

Protein Kinase B (PKB, also known as Akt, or RAC-PK, Related to the A and C protein kinases)[1,3] represents a family of serine/threonine kinases considered to play an important role in the control of cell cycle, cell proliferation, differentiation and in apoptosis. PKB/Akt is the cellular homolog of the viral oncogene *v-akt* of the AKT-8 acute transforming retrovirus found in rodent T cell lymphoma. PKB is composed of an N-terminal pleckstrin-homology (PH) domain, followed by a catalytic kinase domain and a short C-terminal regulatory domain. Three isoforms of PKB have been identified and characterized, PKBα (also termed Akt1, RAC-PKα), PKBβ (Akt2, or RAC-PKβ) and PKBγ.[4,5] PKBα is overexpressed in the breast cancer epithelial cell line MCF7.[2] PKBβ is overexpressed in a significant percentage of ovarian and pancreatic cancers.

PKBα is rapidly activated in response to cell stimulation by several growth factors, insulin, peroxyvanadate or cellular stresses such as heat shock.[6-8] The mechanism of activation and regulation of PKB activity is complex, involving several cellular components. The activation of PKB is mediated through the PI3-kinase signaling pathway and is regulated by PI(3,4,5)P_3-dependent protein kinases (PDKs).[6,7] PI3-kinase activation results in the production of PI(3,4,5)P_3, and PI(3,4)P_2. PKBα appears to bind to PI(3,4)P_2 through its PH domain and translocate to the plasma membrane, where it undergoes dimerization and direct activation by PI(3,4)P_2.[9] Full activation of PKBα requires the phosphorylation of Thr^{308} and Ser^{473} by PDK1 and PDK2, respectively.[10] PKBα appears to regulate the activity of several downstream kinases, including the inhibition of GSK3 and activation of p70 S6 kinase (p70^{s6k}),[6,8] suggesting a role of PKBα in the control of glycogen synthesis, protein synthesis and cell proliferation.

PKB plays a crucial role, in different cell types, as a suppressor of apoptotic cell death induced by a variety of stimuli including growth factor withdrawal, loss of cell adhesion, and DNA damage.[11-16] PKB has been shown to protect cerebellar neurons from apoptosis induced by IGF-1 withdrawal. PKB phosphorylates the Bcl-2 family member BAD at Ser^{136} *in vivo* and *in vitro*, thereby suppressing BAD-induced death and promoting primary neuron survival.[16]

Reagent

Anti-phospho-PKB (pSer^{473}) is supplied as an IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer
Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.
Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2 °C -8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers 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
A minimum working dilution of 1:1,000 is determined by immunoblotting using a whole extract of PDGF-treated mouse fibroblasts NIH3T3 cell line.

A minimum working dilution of 1:1,000 is determined by immunoblotting using a whole extract of H_2O_2-treated rat fibroblasts Rat-1 cell line.

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

References

1. Coffer, P.J., and Woodgett, J.R., Eur. J. Biochem., **201**, 475 (1991).
2. Jones, P.F., et al., Proc. Natl. Acad. Sci. USA, **88**, 4171 (1991).
3. Bellacosa, A., et al., Science, **254**, 274 (1991).
4. Jones, P.F., et al., Cell Regul., **2**, 1001 (1991).
5. Konishi, H. et al., Biochem. Biophys. Res. Commun., **216**, 526 (1995).
6. Burgering, B.M.T., and Coffer, P.J., Nature, **376**, 599 (1995).
7. Franke, T.F., et al., Cell, **81**, 727 (1995).
8. Cross, D.A., et al., Nature, **378**, 785 (1995).
9. Franke, T.F., et al., Science, **275**, 665 (1997).
10. Alessi, D., et al., Curr. Biol., **7**, 261 (1997).
11. Dudek, H., et al., Science, **275**, 661 (1997).
12. Ahmed, N.N., et al., Proc. Natl. Acad. Sci. USA, **94**, 3627 (1997).
13. Kaufmann-Zeh, A., et al., Nature, **385**, 544 (1997).
14. Kulik, G., et al., Mol. Cell. Biol., **17**, 95 (1997).
15. Khwaja, A., et al., EMBO J., **16**, 2783 (1997).
16. Datta, S.R., et al., Cell, **91**, 231 (1997).

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