



## Product Information

### ANTI-PROTEIN KINASE B $\alpha$ (PKB $\alpha$ /Akt1) Developed in Rabbit, IgG Fraction of Antiserum

Product Number **P1601**

#### Product Description

Anti-Protein Kinase B  $\alpha$ /Akt1 (PKB $\alpha$ /Akt1) is developed in rabbit using a synthetic peptide K-DSERRPHFPQF-SYSAS corresponding to the C-terminus of PKB $\alpha$ /Akt1 of human origin (amino acids 462-477) conjugated to BSA as immunogen. This sequence is identical in mouse, rat and bovine PKB $\alpha$ /Akt1, highly conserved in PKB $\beta$ /Akt2, and diverges in PKB $\gamma$ . Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins. The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide (see MSDS)\* as a preservative.

Anti- PKB $\alpha$ /Akt1 may be used for the detection and localization of PKB $\alpha$ /Akt1 by immunoblotting using a whole cell extract of MCF7 cultured cells.

Protein Kinase B (PKB, also known as Akt, or RAC-PK, Related to the A and C protein kinases)<sup>1-3</sup> is a family of serine/threonine kinases considered to play an important role in the control of cell cycle, cell proliferation and differentiation and in apoptosis. Akt is the cellular homologue of the viral oncogene v-akt of the AKT-8 acute transforming retrovirus found in rodent T cell lymphoma. PKB/Akt 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/Akt have been identified and characterized, PKB $\alpha$  (also termed Akt1 or RAC-PK $\alpha$ ), PKB $\beta$  (Akt2, PKB $\beta$  or RAC-PK $\beta$ ),<sup>4</sup> and PKB $\gamma$ .<sup>5</sup> PKB $\alpha$ /Akt1 is overexpressed in the breast cancer epithelial cell line MCF7.<sup>2</sup> /PKB $\beta$ /Akt2 is overexpressed in a significant percentage of ovarian and pancreatic cancers. PKB $\alpha$ /Akt1 is rapidly activated in response to cell stimulation by several growth factors, insulin, peroxyvanadate or by cellular stresses such as heat shock.<sup>6-8</sup> The mechanism of activation and regulation of PKB/Akt activity is complex involving several cellular components. Several lines of evidence indicate that the activation of PKB $\alpha$ /Akt1 is mediated through the PI3-kinase signaling pathway and it is regulated by phosphatidylinositol- 3,4,5-triphosphate dependent protein kinases (PDKs).<sup>6,7</sup> PI3-kinase activation results in the production of the phosphatidyl-

inositolphosphates PtdIns(3,4,5)P<sub>3</sub>, and PtdIns(3,4)P<sub>2</sub>. PKB $\alpha$ /Akt1 appears to bind to PtdIns(3,4)P<sub>2</sub> through its PH domain and to translocate to the plasma membrane, where it undergoes dimerization and direct activation by PtdIns(3,4)P<sub>2</sub>.<sup>9</sup> Full activation of PKB $\alpha$ /Akt1 requires the phosphorylation of Thr<sup>308</sup> by PDK1 and of Ser<sup>473</sup> by PDK2.<sup>10</sup> PKB $\alpha$ /Akt1 appears to regulate the activity of several downstream kinases, including inhibition of GSK3<sup>8</sup> and activation of p70 ribosomal protein S6 kinase (p70<sup>S6k</sup>),<sup>6</sup> suggesting a role of PKB $\alpha$ /Akt1 in the control of glycogen synthesis, protein synthesis and cell proliferation. PKB/Akt plays a crucial role as a suppressor of apoptotic cell death in different cell types, induced by a variety of stimuli including growth factor withdrawal, loss of cell adhesion, and DNA damage.<sup>11-16</sup> PKB/Akt has been shown to protect cerebellar neurons from apoptosis induced by IGF-1 withdrawal. PKB/Akt phosphorylates the Bcl-2 family member BAD at Ser<sup>136</sup> *in vivo* and *in vitro*, thereby suppressing BAD-induced death and promoting primary neuron survival.<sup>16</sup> Antibodies reacting specifically with PKB/Akt isoforms may be used to study the expression and function of these isoforms in a variety of cell types and tissues, and to correlate their expression pattern with physiological functions or pathological conditions.

#### Reagents

Anti-PKB $\alpha$ /Akt1 reacts specifically with PKB $\alpha$ /Akt1 (56 kD) derived from human cell extract. The antibody may be used in immunoblotting of MCF7 cultured cells whole extract. Staining of the PKB $\alpha$ /Akt1 band (56 kD) is specifically inhibited with PKB $\alpha$ /Akt1 peptide (human, amino acids 462-477 with N-terminally added lysine).

#### Precautions and Disclaimer

\* Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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.

Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

#### **Product Profile**

A minimum working dilution of 1: 5,000 is determined by immunoblotting using a whole cell extract of MCF7 cultured cells.

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**

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