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# **ProductInformation**

MONOCLONAL ANTI-PROTEIN KINASE Bα (PKBα/AKT1) CLONE PKB-175 Mouse Ascites Fluid

Product Number P2482

## **Product Description**

Monoclonal Anti-Protein Kinase B $\alpha$  (PKB $\alpha$ /Akt1) (mouse IgG1 isotype) is derived from the PKB-175 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide corresponding to the carboxyterminal sequence (amino acids 461-477) of human PKB $\alpha$ /Akt1, conjugated to KLH. The isotype is determined using Sigma ImmunoType<sup>TM</sup> Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Protein Kinase B $\alpha$  (PKB $\alpha$ /Akt1) recognizes PKB isoform  $\alpha$  (also called Akt1, a 56 kDa molecule) by immunoblotting and ELISA. The epitope recognized by the antibody resides within the C-terminal amino acids 461-477 of human PKB $\alpha$ /Akt1. This sequence is identical in mouse, rat and bovine PKB $\alpha$ /Akt1, is highly conserved in PKB $\beta$ /Akt2 and diverges in PKB $\gamma$ . Reactivity has been observed with human, bovine, rat, mouse and chicken PKB $\alpha$ /Akt1.

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, PKBa (also termed Akt1, 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> Akt2/PKBβ is overexpressed in a significant percentage of ovarian and pancreatic cancers. PKBa/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 phosphatidylinositolphosphates PtdIns(3,4,5)P<sub>3</sub>, and PtdIns(3,4)P<sub>2</sub>. PKBα/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 PKBa/Akt1 requires the phosphorylation of Thr<sup>308</sup> by PDK1 and of Ser<sup>473</sup> by PDK2.<sup>10</sup> PKBα/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 PKB/Akt isoforms in a variety cell types and tissues, and to correlate their expression pattern with physiological functions or pathological conditions.<sup>8</sup>

## Reagents

Monoclonal Anti-Protein Kinase B $\alpha$  (PKB $\alpha$ /Akt1) is supplied as ascites fluid 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 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. Working dilution samples should be discarded if not used within 12 hours.

## **Product Profile**

A minimum working dilution of 1:5,000 is determined by immunoblotting using cultured human breast adenocarcinoma MCF-7 cells.

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

### References

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