

**ANTI-PDK1**  
**Developed in Rabbit**  
**IgG Fraction of Antiserum**Product Number **P 3110****Product Description**

Anti-PDK1 is developed in rabbit using a synthetic peptide corresponding to the C-terminus of human PDK1 (amino acids 538-556) conjugated to KLH as immunogen. This sequence is highly conserved (90% homology) in rat and mouse PDK1. 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.

Anti-PDK1 recognizes human PDK1 (60 kDa). Applications include the detection of PDK1 by immunoblotting. Staining of PDK1 in immunoblotting is specifically inhibited with PDK1 immunizing peptide (human, amino acids 538-556).

PDK1 (3-phosphoinositide-dependent protein kinase-1, also known as PKB kinase) is a serine/threonine protein kinase acting upstream of protein kinase B (PKB/Akt1) which is involved in the control of proliferation, metabolism, and apoptosis.<sup>1-4</sup> The mechanism of activation and regulation of PKB activity is complex involving several cellular components. PKB is rapidly activated in response to cell stimulation by several growth factors, insulin, peroxyvanadate, or by cellular stresses such as heat shock.<sup>5-7</sup> The activation of PKB is mediated through the PI3-kinase signaling pathway.<sup>5-8</sup> PI3-kinase activation results in the production of lipid metabolites phosphatidylinositol-3,4,5-triphosphate PI(3,4,5)P<sub>3</sub> and phosphatidylinositol-3,4-diphosphate PI(3,4)P<sub>2</sub>. These lipids bind to PKB through its amino terminal pleckstrin (PH) domain and induce a conformational change in the protein kinase, resulting in the translocation to the plasma membrane.<sup>9,10</sup> Following cell activation, PKB undergoes phosphorylation in the presence of PI(3,4,5)P<sub>3</sub>, by two different protein kinases, PDK1 which phosphorylates Thr<sup>308</sup> in the activation loop of the kinase domain of PKB, and PDK2 which phosphorylates Ser<sup>473</sup> near the carboxyl terminal of PKB.<sup>1, 3, 7</sup>

PDK1 is a constitutively active kinase that is neither stimulated by insulin nor is dependent on PI3-kinase activity in the cell. It contains a PH domain that binds tightly PI(3,4,5)P<sub>3</sub>. However, unlike PKB, its kinase activity is not dependent on binding of PI(3,4,5)P<sub>3</sub>, nor

**Product Information**

inhibited by PI3-kinase inhibitors. PDK1 appears to be localized to a large extent in the cytoplasm, and to some extent at the plasma membrane as a result of its binding to PI(3,4,5)P<sub>3</sub>. PDK can phosphorylate and activate several other serine/threonine protein kinases, including PKC, p70<sup>S6k</sup> and the serum and glucocorticoid-regulated protein kinase (SGK), via a PI3-kinase dependent pathway.<sup>11-14</sup> PDK1 specifically phosphorylates p70<sup>S6k</sup> at Thr<sup>229</sup>. PDK2 phosphorylates SGK at Ser<sup>422</sup>, followed by a PI3-kinase-independent phosphorylation at Thr<sup>256</sup> by PDK1, which activates SGK.

**Reagent**

Anti-PDK1 is supplied as a solution 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 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 prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is also 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 recombinant human PDK-1.

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

**References**

1. Alessi, D.R., et al., *Curr. Biol.*, **7**, 261 (1997).
2. Alessi, D.R., et al., *Curr. Biol.*, **7**, 776 (1997).
3. Stokoe, D., et al., *Science*, **277**, 567 (1997).

4. Stephens, L., et al., Science, **279**, 710 (1998).
5. Burgering, B.M.T., and Coffey, P.J., Nature, **376**, 599 (1995).
6. Cross, D.A., et al., Nature, **378**, 785 (1995).
7. Marte, B.M., and Downward, J., Trends Biochem. Sci., **22**, 355 (1997).
8. Banfic, H., et al., J. Biol. Chem., **273**, 11630 (1998).
9. Klippel, A., et al., Mol. Cell. Biol., **17**, 338 (1997).
10. Andjelkovic, M., et al., J. Biol. Chem., **272**, 31515 (1997).
11. Belham, C., et al., Curr. Biol., **9**, R93 (1999).
12. Pullen, N., et al., Science, **279**, 707 (1998).
13. Le Good, J.A., et al., Science, **281** 2042, (1998).
14. Park, J., et al., EMBO J., **18**, 3024 (1999).

ER/KAA 01/02

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.