

## Product Information

### ANTI- PROTEIN KINASE D (PKD/PKC $\mu$ )

Developed in Rabbit, IgG Fraction of Antiserum

Product Number **P 3987**

#### Product Description

Anti-Protein Kinase D (PKD/ PKC $\mu$ ) is developed in rabbit using a synthetic peptide corresponding to the C-terminal sequence of human PKD/PKC $\mu$  (amino acids 893-912 with N-terminally added lysine) conjugated to KLH as immunogen. This sequence is highly conserved (80% homology) in mouse PKD/PKC $\mu$ . 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-Protein Kinase D (PKD/PKC $\mu$ ) recognizes human and mouse PKD/PKC $\mu$  (110 kDa). Applications include the detection of PKD/PKC $\mu$  by immunoblotting and immunoprecipitation. Staining of PKD/PKC $\mu$  in immunoblotting is specifically inhibited with the PKD/PKC $\mu$  immunizing peptide.

Protein Kinase D (PKD, also named protein kinase C $\mu$ ) is a serine/threonine protein kinase related to the family of protein kinase C (PKC).<sup>1-3</sup> PKD/PKC $\mu$  (110 kDa) contains a N-terminal cysteine-rich, zinc finger-like domain that binds diacylglycerol (DAG) and phorbol esters with high affinity, but lacks the C2 calcium binding domain found in the classical PKCs. In contrast to other PKCs, the N-terminal regulatory region of PKD/PKC $\mu$  contains a pleckstrin homology (PH) domain that regulates enzymatic activity and lacks a sequence with homology to a typical PKC autoinhibitory pseudosubstrate motif.<sup>4</sup> The catalytic PKD/PKC $\mu$  domain shows little similarity to the highly conserved regions of the kinase subdomains of the PKC family, instead it shows homology to that of Ca<sup>2+</sup>-regulated kinases.

PKD/PKC $\mu$  does not phosphorylate a variety of substrates utilized by PKCs *in vitro*, but shows optimal activity for a unique peptide substrate, indicating that PKD/PKC $\mu$  is a protein kinase with distinct substrate specificity.<sup>2,5</sup> PKD/PKC $\mu$  can be activated by pharmacological agents such as phorbol esters and bryostatin1 and by a variety of physiological stimuli, such as PDGF, TNF $\alpha$ , angiotensin-II and neuropeptide agonists, and oxidative stress.<sup>3,6-10</sup>

PKD/PKC $\mu$  appears to play a role in Golgi structure and function.<sup>11</sup> It may also serve as a molecular switch to promote cell proliferation while inhibiting apoptosis.<sup>12,13</sup> PKD/PKC $\mu$  can be activated by DAG or phorbol esters in the presence of phosphatidylserine. The activation of PKD/PKC $\mu$  *in-vivo* is regulated by multisite phosphorylation events. These include phosphorylation of Ser<sup>203</sup> in the regulatory domain and Ser<sup>255</sup> after *in vivo* stimulation with phorbol esters, Ser<sup>744</sup> and Ser<sup>748</sup> in the activation loop, and phosphorylation of the C-terminal Ser<sup>916</sup>.<sup>14-16</sup> The phosphorylation of these residues, and subsequent PKD/PKC $\mu$  activation, is not an autophosphorylation event but is mediated by a novel PKC-dependent signaling pathway. In particular, the PKC $\epsilon$  and PKC $\eta$  isoforms have been implicated in the regulation of PKD/PKC $\mu$  activity.<sup>17</sup> PKC $\eta$  can interact directly with the PH domain of PKD/PKC $\mu$ , indicating a direct link between PKC $\eta$  and PKD/PKC $\mu$ .

#### Reagent

Anti-Protein Kinase D (PKD/PKC $\mu$ ) 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 to 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 1:500 is determined by immunoblotting using a whole cell extract of the human epidermal carcinoma A431 cell line.

For immunoprecipitation, the antibody immunoprecipitates PKD/PKC $\mu$  from a lysate of cultured NIH3T3 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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