

## Product Information

### Monoclonal Anti-Phospho DAP-Kinase (pSer<sup>308</sup>)

Clone DKPS308

Purified Mouse Immunoglobulin

Product Number **D 4941**

#### Product Description

Monoclonal Anti-Phospho DAP-Kinase (pSer<sup>308</sup>) (mouse IgG1 isotype) is derived from the DKPS308 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a phospho peptide corresponding to amino acids 303-312 (pSer<sup>308</sup>) of human DAP-kinase, conjugated to KLH. The isotype is determined using Sigma ImmunoType<sup>™</sup> Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Phospho DAP Kinase (pSer<sup>308</sup>) recognizes human DAP-Kinase phosphorylated at Ser<sup>308</sup> (approx. 160 kDa). The product may be used in ELISA, immunoprecipitation, and immunoblotting.

Various apoptotic signals, including interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , Fas, and activation of c-myc, use Death Associated Protein Kinase (DAPK) as a downstream effector in different cell types. DAPK is a positive mediator of apoptosis and is widely expressed in many tissues of embryonic and adult origin.<sup>1-3</sup>

DAPK is a Ca<sup>2+</sup>/calmodulin-dependent Ser/Thr kinase that associates with microfilaments. The protein is composed of a multidomain structure. It has a subdomain typical of serine/threonine kinases, a Ca<sup>2+</sup>/calmodulin regulatory domain, eight ankyrin repeats followed by two P-loop motifs and a typical death domain module. It contains two auto-inhibitory domains one of them Ca<sup>2+</sup>/calmodulin dependent. In the absence of this latter domain, DAPK is constitutively active.<sup>2-5</sup>

DAPK activity is also regulated by phosphorylation. DAPK was found to be negatively regulated by autophosphorylation at Ser<sup>308</sup> which is in the calmodulin regulatory domain.<sup>6</sup> This autophosphorylation, which occurs in cells at the basal state, lowers the affinity of DAPK for calmodulin and thus the kinase is inactive. Under some apoptotic conditions DAPK undergoes dephosphorylation. As a consequence, it binds to calmodulin with higher affinity, becomes activated, phosphorylates its downstream substrate proteins, and mediates apoptosis.<sup>6</sup>

Monoclonal antibodies specific for Phospho DAP-Kinase (pSer<sup>308</sup>) are important tools for studying the mechanism of DAPK activation in apoptosis.

#### Reagent

Monoclonal Anti-Phospho DAP Kinase (pSer<sup>308</sup>) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

#### 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 hazards 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 dilutions should be discarded if not used within 12 hours.

**Product Profile**

A minimum working antibody concentration of 1-2  $\mu$ g/ml is determined by immunoblotting using 293T (human embryonal kidney) cells transfected with DAP-Kinase expression vector.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

**References**

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5. Raveh, T., et al., *Proc. Natl. Acad. Sci. USA*, **97**, 1572-1577 (2000).
6. Shoat, G., et al., *J. Biol. Chem.*, **276**, 47460-47467 (2001).

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