

DAPK3 Kinase

☑ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full-length human DAPK3 (Met1-Arg454) kinase, supplied as a GST fusion protein.

Background: Death-associated protein kinase (DAPK) is a Ca²⁺/calmodulin-regulated serine/threonine kinase that participates in a wide range of apoptotic signals including interferon- γ , tumor necrosis factor- α , Fas, activated c-Myc, and detachment from the extracellular matrix. In addition to the kinase domain and calmodulin regulatory segment, DAPK also has eight ankyrin repeats, a cytoskeleton binding region, and a conserved death domain (1,2,3). Deletion of the calmodulin-regulatory domain generated a constitutively active mutant kinase. Ectopic expression of wild-type DAPK induced cell death in HeLa cells. Conversely, expression of a catalytically inactive mutant protected cells from interferon-gamma-induced cell death (4). The catalytic domain of DAPK has very high sequence similarity to vertebrate myosin light chain kinase (MLCK) and a RXX(S/T)X motif derived from myosin light chain protein was shown to be phosphorylated *in vitro* by DAPK (5).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human DAPK3 (Met1-Arg454) (GenBank Accession No. NM_001348) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

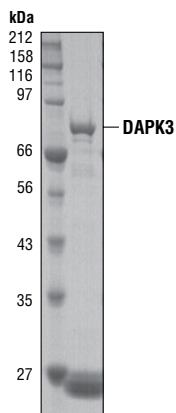


Figure 1. The purity of the DAPK3 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-DAPK3 protein is 79 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. DAPK3 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Kimchi, A. (1999) *Ann Rheum Dis* 58, 114–119.
- (2) Cohen, O. et al. (1999) *J Cell Biol* 146, 141–148.
- (3) Deiss, L. P. et al. (1995) *Genes Dev* 9, 15–30.
- (4) Cohen, O. et al. (1997) *EMBO J* 16, 998–1008.
- (5) Velentza, A. V. et al. (2001) *J Biol Chem* 276, 38956–38965.

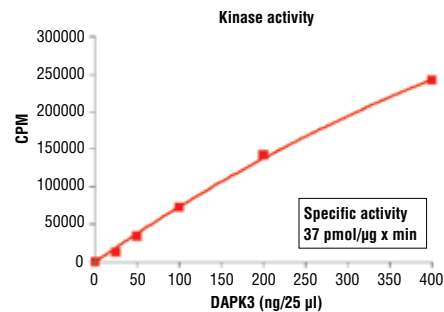


Figure 2. DAPK3 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl₂, 0.05 mM DTT, 50 μ M ATP, Substrate: MBP 200 ng/ μ l, and variable amounts of recombinant DAPK3.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for DAPK3 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. Kinase Buffer (5X)

25 mM MOPS, pH 7.2
12.5 mM β -glycerophosphate
5 mM EGTA
2 mM EDTA
25 mM $MgCl_2$
0.25 mM DTT

2. ATP (10 mM) #9804

3. ^{32}P - γ ATP

4. MBP (0.5 μ g/ μ l)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer by 1:40 to make 250 μ M ATP.
2. Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute DAPK3 protein (100 ng/ μ l concentration) to 40 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 μ l diluted DAPK3 kinase solution, 10 μ l MBP (0.5 μ g/ μ l), and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

5 mM MOPS, pH 7.2
2.5 mM β -glycerophosphate
1 mM EGTA
5 mM $MgCl_2$
0.05 mM DTT
50 μ M ATP
200 ng/ μ L MBP

6. After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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