

DAPK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # D01-11G

Lot # A1484-4

Product Description

Recombinant human DAPK1 (1-363) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_004938](#).

Gene Aliases

DAPK, DKFZp781I035

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

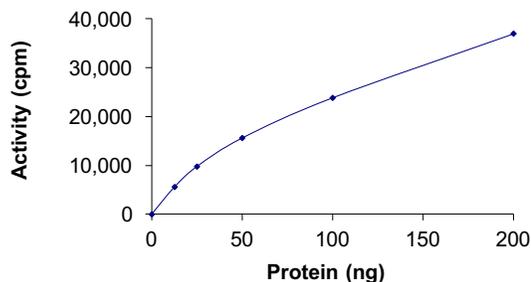
Scientific Background

Death-associated protein kinase 1 (DAPK1) is a positive mediator of apoptosis induced by γ -interferon. Activation of DAPK occurs via dephosphorylation of Ser-308 and subsequent association of calcium/calmodulin (1). DAPK is rapidly dephosphorylated in response to tumor necrosis factor or ceramide and then subsequently degraded via proteasome activity. The decline in DAPK expression is paralleled with increased caspase activity and cell apoptosis. Studies suggest that the apoptosis regulatory activities mediated by DAPK are controlled both by phosphorylation status and protein stability (2).

References

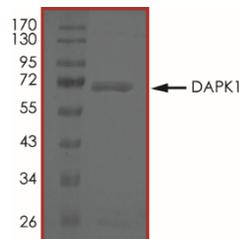
1. Deiss, L. P. et al: Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. *Genes Dev.* 1995; 9: 15-30.
2. Feinstein, E. et al: Assignment of DAP1 and DAPK: genes that positively mediate programmed cell death triggered by IFN-gamma--to chromosome regions 5p12.2 (sic) and 9q34.1, respectively. *Genomics.* 1995; 29: 305-307.

Specific Activity



The specific activity of DAPK1 was determined to be **42 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. DAPK1 Approx. MW **71kDa**.

DAPK1, Active

Recombinant protein expressed in Sf9 cells

Catalog # D01-11G
Specific Activity 42 nmol/min/mg
Lot # A1484-4
Purity >90%
Concentration 0.1 µg/µl
Stability 1yr at -70°C from date of shipment
Storage & Shipping Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: D01-11G)

Active DAPK1 (0.1 µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active DAPK1 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100 µl [³³P]-ATP (1mCi/100 µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 µl aliquots at -20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active DAPK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µl:
 - Component 1.** 10 µl of diluted Active DAPK1 (Catalog #D01-11G)
 - Component 2.** 5 µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 2.5 µl of Ca²⁺/Calmodulin solution (10X) (Catalog #C02-39)
 - Component 4.** 2.5 µl distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25 µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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