550-5600 Parkwood Way
Richmond, BC V6V2M2
CANADA
Tel: 1-(604)-232-4600
Fax: 1-(604)-232-4601
orders@signalchem.com

DAPK3, Active

Full-length Recombinant protein expressed in Sf9 cells

Catalog # D03-10G
Lot # G359-2

Product Description

Full-length recombinant human DAPK3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001348.

Gene Aliases

ZIP, ZIPK, FLJ36473

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

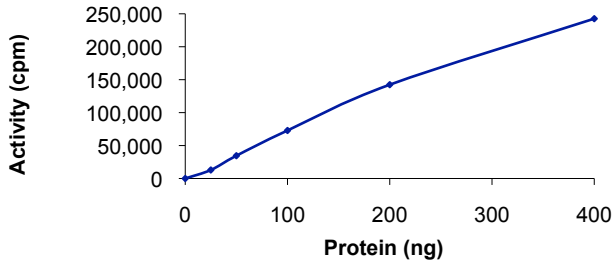
Scientific Background

DAPK3 or Death-associated protein kinase 3 (also known as ZIP) plays a role in apoptosis (1). DAPK3 is a nuclear serine/threonine-specific kinase that phosphorylates core histones H3 and H4, and myosine light chain *in vitro*. DAPK3 interacts with transcription and splicing factors as well as with pro-apoptotic protein Par-4 suggesting that it participates in multiple cellular processes. DAPK3 contains a leucine zipper structure at its C terminus and this region is responsible for binding to ATF4. The leucine zipper domain is necessary for the homodimerization of DAPK3 as well as for the activation of the kinase (2).

References

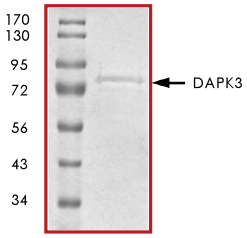
1. Kawai, T. et al: ZIP kinase, a novel serine/threonine kinase which mediates apoptosis. Mol. Cell Biol. 1998;18(3):1642-51.
2. Preuss, U. et al. Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase. Nucleic Acids Res. 2003; 31(3):878-85.

Specific Activity



The specific activity of DAPK3 was determined to be **37 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>80%** by densitometry.
DAPK3 Approx. MW **79kDa**.

DAPK3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	D03-10G
Specific Activity	37 nmol/min/mg
Specific Lot Number	G359-2
Purity	>80%
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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: D03-10G)

Active DAPK3 (0.1μg/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 $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 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 200μl aliquots at –20°C.

Substrate (Catalog #: M42-54G)

Myelin Basic Protein (MBP) diluted in distilled H_2O to a final concentration of 0.2mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active DAPK3, 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 20μl:

- **Component 1.** 10μl of diluted Active DAPK3 (Catalog #D03-10G)
- **Component 2.** 5μl of 0.2mg/ml stock solution of substrate (Catalog #M42-54G)
- **Component 3.** 5μl distilled H_2O (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 H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl 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 20μl 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 H_2O) 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

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

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

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

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