DAPK2, Active
Full-length recombinant protein expressed in Sf9 cells

Catalog # D02-10G
Lot # H031-2

Product Description
Recombinant human full-length DAPK2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_014326.

GeneAliases
DRP-1; MGC119312

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 & 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
DAPK2 or death-associated protein kinase 2 belongs to a family of proapoptotic Ca(2+)/calmodulin-regulated serine/threonine kinases. Overexpression of DAPK2 induces cell apoptosis. DAPK2 has been shown to be a novel Sp1-dependent target gene for E2F1 and Krüppel-like factor 6 (KLF6) in cell death response (1). Both E2F1 and KLF6 strongly activate the DAPK2 promoter. DAPK2 plays a role in granulopoiesis where it is highly expressed. β-catenin can block anoikis of malignant kidney and intestinal epithelial cells and promote their anchorage-independent growth by down-regulating DAPK2 (2). β-catenin-induced down-regulation of DAPK2 requires the presence of the transcription factor TCF-4.

References

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: D02-10G)
Active DAPK2 (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 DAPK2 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.

Substrate (Catalog #: M89-54G)
LC20 protein substrate, 0.2mg/ml concentration.

[33P]-ATP Assay Cocktail
Prepare 250µM [33P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [33P]-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.

Assay Protocol

Step 1. Thaw [33P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active DAPK2, 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 DAPK2 (Catalog #D02-10G)
  Component 2. 5µl of 0.2mg/ml stock solution of substrate (Catalog #M89-54G)
  Component 3. 2.5µl of Ca²⁺ / Calmodulin Solution II, 10x (Catalog #C02-39B)
  Component 4. 2.5µl of 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 5µl [33P]-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 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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5µl [³³P]-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 ³³P-ATP in cpm/pmol]*[Reaction time in min]*[Enzyme amount in µg or mg])*([Reaction Volume) / (Spot Volume)]

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