

## DAPK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # D01-11G-10

Lot # V250-1

### 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, DKFzp7811035

### Concentration

0.1 µg/µl

### 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, Shipping 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. Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

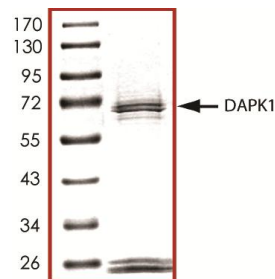
### Scientific Background

Death-associated protein kinase 1 (DAPK1) is a positive mediator of apoptosis induced by  $\gamma$ -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.

### Purity

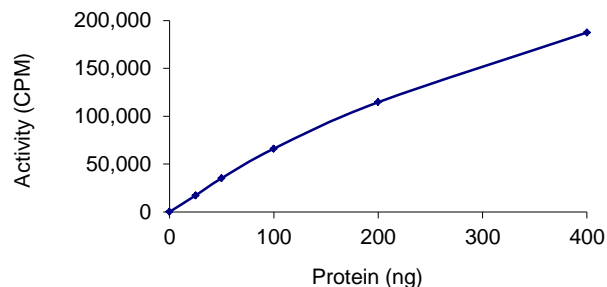


**Figure 1. SDS-PAGE gel image**

The purity of DAPK1 was determined to be **>70%** by densitometry, approx. MW **71kDa**.

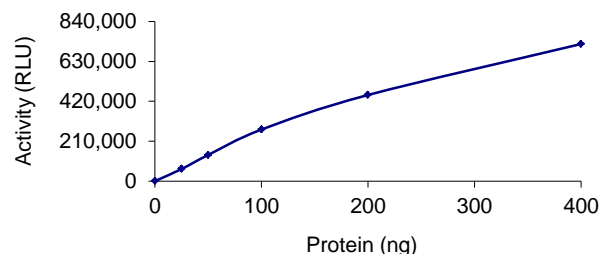
### Specific Activity

**Figure 2. Radiometric Assay Data**



The specific activity of DAPK1 was determined to be **42 nmol /min/mg** as per activity assay protocol. (For Radiometric Assay Protocol on this product please see pg. 2)

**Figure 3. ADP-Glo™ Assay Data**



The specific activity of DAPK1 was determined to be **50 nmol /min/mg** as per activity assay protocol. (For ADP-Glo™ Assay Protocol on this product please see pg. 3)

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: D01-11G-10)

Active DAPK1 (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 MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>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 [<sup>33</sup>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 #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-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 20µl:
  - Component 1.** 10µl of diluted Active DAPK1 (Catalog #D01-11G-10)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
  - Component 3.** 2.5µl of Ca<sup>2+</sup>/Calmodulin solution (10X) (Catalog #C02-39)
  - Component 4.** 2.5µl distilled H<sub>2</sub>O (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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>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<sub>2</sub>O) 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 (cpm) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>33</sup>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 <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

# ADP-Glo™ Activity Assay Protocol

## Reaction Components

### DAPK1 Kinase Enzyme System (Promega, Catalog #:V4096)

DAPK1, Active, 10µg (0.1µg/µl)  
MBP Protein, 1ml (1mg/ml)  
Reaction Buffer A (5X), 1.5ml  
DTT (0.1M), 25µl  
Ca<sup>2+</sup>/Calmodulin Solution, 10X, 500µl

### ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP solution, 10 mM (0.5ml)  
ADP solution, 10 mM (0.5ml)  
ADP-Glo™ Reagent (5ml)  
Kinase Detection Buffer (10ml)  
Kinase Detection Substrate (Lyophilized)

### Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl<sub>2</sub> and 0.5 mg/ml BSA.

## Assay Protocol

The DAPK1 assay is performed using the DAPK1 Kinase Enzyme System (Promega; Catalog #: V4096) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The DAPK1 reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html).

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of DAPK1 Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH<sub>2</sub>O.
- Step 4.** Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH<sub>2</sub>O.
- Step 5.** Prepare diluted DAPK1 in 1X Buffer (diluted from 2X buffer) 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).
- Step 6.** In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

<b>Component 1.</b>	5µl of diluted Active DAPK1
<b>Component 2.</b>	5µl of 1 mg/ml stock solution of substrate
<b>Component 3.</b>	2.5µl of Ca <sup>2+</sup> /Calmodulin solution (10X)
<b>Component 4.</b>	7.5µl of 2X Buffer

- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 8.** At the same time as the DAPK1 kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the ADP-Glo™ Kinase Assay technical Manual #TM313.
- Step 9.** Initiate the DAPK1 reactions by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® plate reader (Promega; Cat# E7031).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLU, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

### Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol / (Reaction time in min)\*(Enzyme amount in mg)