

ZAK, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # Z01-10G

Lot # S084-3

Product Description

Recombinant full-length human ZAK was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_133646](#).

Gene Aliases

AZK, MLT, MRK, MLK7, MLTK, mlklak, MAP3K20

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.

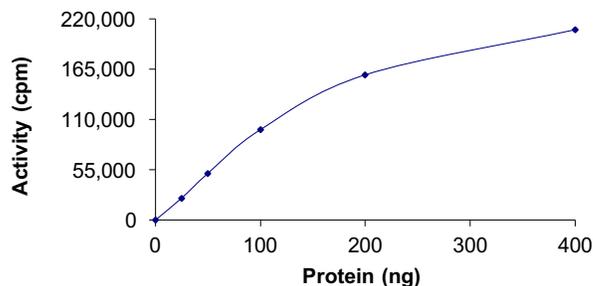
Scientific Background

ZAK is a member of the MAPKKK family of signal transduction molecules and mediates gamma radiation signaling leading to cell cycle arrest. The activity of ZAK plays a role in cell cycle checkpoint regulation as well as being involved in regulating actin organization (1). Expression of kinase-dead ZAK in mouse fibroblasts disrupts actin stress fibers and causes morphologic changes. ZAK can activate JNK through MKK4/MKK7 and ERK5/p38-gamma via MKK3/MKK6. Expression of ZAK increases the population of cells in the G2/M phase of the cell cycle, whereas dominant-negative ZAK attenuated the G2 arrest caused by gamma radiation (2).

References

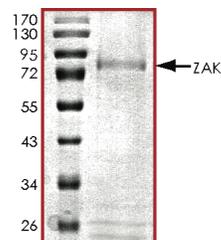
1. Yang J-J, et al: Mixed lineage kinase ZAK utilizing MKK7 and not MKK4 to activate the c-Jun N-terminal kinase and playing a role in the cell arrest. *Biochem. Biophys. Res. Commun.* 297: 105-110, 2002.
2. Gross E. A, et al: MRK, a mixed lineage kinase-related molecule that plays a role in gamma-radiation-induced cell cycle arrest. *J. Biol. Chem.* 277: 13873-13882, 2002.

Specific Activity



The specific activity of ZAK was determined to be **60 nmol/min/mg** as per activity assay protocol.

Purity



The purity of ZAK was determined to be **>75%** by densitometry, approx. MW **~82kDa**.

ZAK, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog Number	Z01-10G
Specific Activity	60 nmol/min/mg
Specific Lot Number	S084-3
Purity	>75%
Concentration	0.05 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 #: Z01-10G)

Active ZAK (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 ZAK 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 50ng/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 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.

Substrate (Catalog #: M42-51N)

Myelin Basic Protein (MBP) substrate 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 ZAK, 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 ZAK (Catalog #Z01-10G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5µl 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 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 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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