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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
A05-11G-05	5 µg
A05-11G-10	10 µg

ACK, Active

Recombinant human protein expressed in Sf9 cells

Catalog # A05-11G
Lot # T990-2

Product Description

Recombinant human ACK (110-476) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005781.

Gene Aliases

TNK2, ACK1, FLJ44758, FLJ45547, p21cdc42Hs

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.

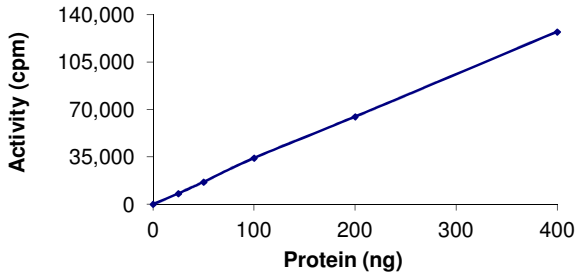
Scientific Background

ACK is a tyrosine kinase that binds CDC42Hs in its GTP-bound form and inhibits both the intrinsic and GTPase-activating protein (GAP)-stimulated GTPase activity of CDC42Hs (1). Overexpression of ACK in cancer cell lines of epithelial origin increases cellular motility and invasiveness. In a mouse model, ACK overexpression enhances the ability of a human breast cancer cell line to metastasize to the lung and increased mortality (2). Ligand stimulation of alpha-3 beta-1 integrin leads to activation of ACK which then enhances p130CAS phosphorylation and activation of RAC.

References

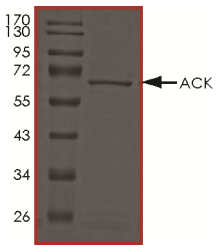
1. Manser, E. et al: A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42. Nature 363: 364-367, 1993.
2. van der Horst, E. H.; Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. Proc. Nat. Acad. Sci. 102: 15901-15906, 2005.

Specific Activity



The specific activity of ACK was determined to be **21 nmol /min/mg** as per activity assay protocol.

Purity



The purity of ACK was determined to be **>95%** by densitometry, approx. MW **66kDa**.

ACK, Active

Recombinant protein expressed in Sf9 cells

Catalog #	A05-11G
Specific Activity	21 nmol/min/mg
Lot #	T990-2
Purity	>95%
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 #: A05-11G)

Active ACK (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 ACK for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 12.5mM $MnC1_2$, 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 [^{33P}]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). 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 II (Catalog #: K02-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: P61-58)

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H_2O to a final concentration of 1 mg/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 ACK, 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 ACK (Catalog #A05-11G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #P61-58)
- **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 5 µl [^{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 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 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 5 µl [^{33}P]-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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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