

Catalogue #	Aliquot Size
A05-11G-05	5 µg
A05-11G-10	10 µg
A05-11G-20	20 µg

ACK, Active

Recombinant protein expressed in Sf9 cells

Catalog # A05-11G

Lot # H007-1

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.

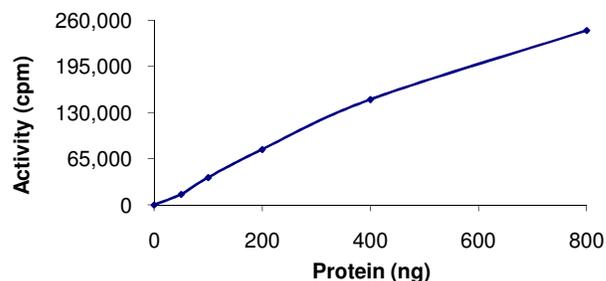
Scientific Background

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

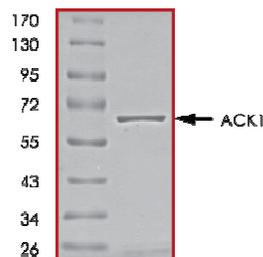
- Manser, E. et al: A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42. *Nature* 363: 364-367, 1993.
- van der Horst, E. H.; Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. *Proc. Nat. Acad. Sci.* 102: 15901-15906, 2005.

Specific Activity



The specific activity of ACK was determined to be **20 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 Number A05-11G
Specific Activity 20 nmol/min/mg
Specific Lot Number H007-1

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 MgCl₂, 12.5mM MnCl₂, 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 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

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1 mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-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
 - 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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