

Ack1 Kinase

✓ 5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant human Ack1 (Gly110-Trp476) kinase, supplied as a GST fusion protein.

Background: Acks (activated cdc42-associated kinases) are non-receptor tyrosine kinases that comprise a tyrosine kinase core, an SH3 domain, a cdc42-binding region, a Ralt homology region and a proline-rich region (1,2). So far, 2 Acks, Ack1 and Ack2 have been identified. They are the only 2 tyrosine kinase known to interact with cdc42. Both Acks are activated by stimulations including EGF, PDGF and activation of integrins by cell adhesion and may serve as a point of convergence between receptor tyrosine kinase or G protein-coupled receptor signaling and cdc42. Acks may regulate cell growth, morphology and motility (3,4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Ack1 (Gly110-Trp476) (GenBank Accession No. NM_005781.4) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

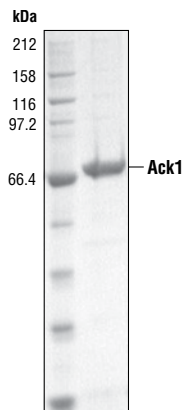


Figure 1. The purity of the GST-Ack1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-Ack1 fusion protein is 71 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Ack1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Galisteo, M.L. et al. (2006) *Proc. Natl. Acad. Sci. USA* 103, 9796–9801.
- (2) Yokoyama, N. and Miller, W.T. (2003) *J. Biol. Chem.* 278, 47713–47723.
- (3) Yang, W. and Cerione, R.A. (1997) *J. Biol. Chem.* 272, 24819–24824.
- (4) Yang, W. et al. (2001) *J. Biol. Chem.* 276, 43987–43993.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Serine/Threonine Kinase Substrate Screening Kit #7400

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

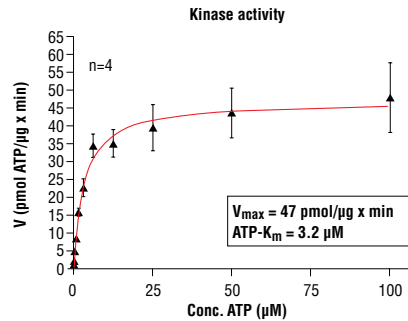


Figure 2. Ack1 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 3 µM Na-orthovanadate, 1.2 mM DTT, 2.5 µg/50 µl PEG20,000, ATP variable, Substrate: Poly (EY: 4:1) 20 ng/µL and recombinant Ack1: 200 ng/50 µl.

Protocol for Ack1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (5X)**
300 mM HEPES-NaOH, pH 7.5
15 mM MgCl₂
15 mM MnCl₂
15 μM Na-orthovanadate
6 mM DTT
12.5 μg/50 μl PEG_{20,000}
- 2. ATP (10 mM) #9804**
- 3. ³²P-γATP**
- 4. Poly(EY, 4:1) (100 ng/μl)**

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μM ATP.
- 2.** Dilute [³²p] ATP to 0.16 μCi/μl [³²p] ATP with 250 μM ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute Ack1 kinase protein (635 ng/μl concentration) to 20 ng/μl with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μl diluted Ack kinase solution, 10 μl Poly(EY, 4:1) (100 ng/μl), and 5 μl 0.16 μCi/μl [³²p] ATP solution.

Final Assay Conditions

- 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 μM Na-orthovanadate
 - 1.2 mM DTT
 - 2.5 μg/50 μl PEG_{20,000}
 - 20 ng/μL Poly(EY, 4:1)
- 6.** After 15 minutes terminate reaction by spotting 20 μl of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.