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ITK, Active

Recombinant protein expressed in Sf9 cells

Catalog # I13-11G

Lot # P167-1

Product Description

Recombinant human ITK (352-end) was expressed in Sf9 cells using an N-terminal GST tag. The gene accession number is NM_005546.

Gene Aliases

EMT; LYK; PSCTK2; MGC126257; MGC126258

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

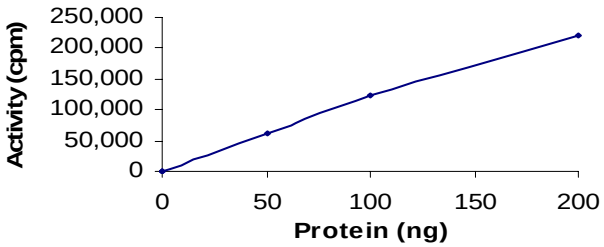
Scientific Background

ITK is a member of the TEC family of non-receptor tyrosine kinases. ITK is expressed in T-cells and is important for T-cell development and activation through the antigen receptor. ITK require prior activation of Lck, Zap-70 and PI3-kinase for efficient activation and shares major substrates with both Lck and Zap-70 (1). ITK knockout mice show multiple effects on T cell development, cytokine production and T-helper cell differentiation. T cells that lack or express mutant versions of ITK show impaired TCR-induced actin polymerization, cell polarization and regulation of the signaling events involved in cytoskeletal reorganization (2).

References

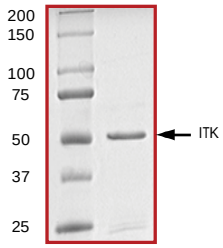
1. August, A. et al: The Tec family of tyrosine kinases in T cells, amplifiers of T cell receptor signals. Int J Biochem Cell Biol. 2002 Oct;34(10):1184-9.
2. Finkelstein, L D. et al: Tec kinases: shaping T-cell activation through actin. Trends Cell Biol. 2004 Aug;14(8):443-51.

Specific Activity



The specific activity of ITK was determined to be **58 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry.
Approx. MW **~53kDa**.

ITK, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	I13-11G
Specific Activity	58 nmol/min/mg
Specific Lot Number	P167-1
Purity	>90%
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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: I13-11G)

Active ITK (0.1μg/μl) diluted with Kinase Dilution Buffer (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 ITK for optimal results).

Kinase Dilution Buffer, pH 7.2 (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, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}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 [^{32}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer (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 (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate

Myelin basic protein (MBP) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active ITK, Kinase Assay Buffer, Substrate and Enzyme 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 ITK (Catalog # I13-11G).
- **Component 2.** 10μl of 1 mg/ml stock solution of substrate

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 5μl [^{32}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_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^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{32}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 ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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