Recombinant Human Active ITK aa 352-620

Catalog Number: 4587-KS

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 9 (baculovirus)-derived aa 352-620 Accession # NM_005546
N-terminal Sequence Analysis	Using an N-terminal GST tag

SPECIFICATIONS

SDS-PAGE	53 kDa
Activity	The activity of ITK is typically 49-67 nmol/min/mg using a myelin basic protein (MBP) substrate (see Activity Assay Protocol).
Purity	>70%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	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, and 25% glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Active Kinase - Active ITK (0.1 µg/µL) diluted with Kinase Dilution Buffer. Note: These are suggested working dilutions. *Optimal dilutions should be determined by each laboratory for each application.*
- Kinase Assay Buffer I, pH 7.2 - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- Kinase Dilution Buffer, pH 7.2 - Kinase Assay Buffer I diluted 5-fold with a 50 ng/µL BSA solution.
- 10 mM ATP Stock Solution - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 µL aliquots at ≤ -20° C.
- [^{33}P]-ATP Assay Cocktail - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [^{33}P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20° C.
- Substrate - Myelin Basic Protein (MBP) substrate diluted in distilled or deionized water to a final concentration of 1 mg/mL.

Assay

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active ITK, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.
   a. Diluted Active ITK: 10 µL
   b. MBP Substrate (1 mg/mL; on ice): 5 µL
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 or deionized water.
5. Initiate the reaction with the addition of 5 µL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter
9. Determine the corrected cpm by subtracting the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below:

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)
Specific Activity (SA) = cpm for 5 µL [^{33}P]-ATP/pmol of ATP (in 5 µL of a 250 µM ATP stock solution; *i.e.* 1250 pmol)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/µg or nmol/minutes/mg)
Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	This product is stable at ≤ -70° C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

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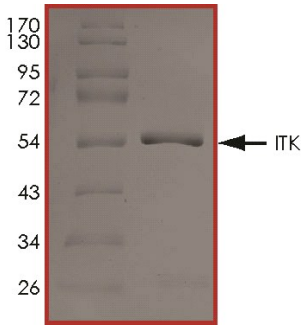
DATA

SDS-PAGE



The approximate molecular weight is 53 kDa and the average purity is 90%.

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 requires prior activation of Lck, ZAP70, and PI3-kinase for efficient activation and shares major substrates with both Lck and ZAP70 (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.* (2002) Int. J. Biochem. Cell Biol. **34**:1184.
2. Finkelstein, L.D. *et al.* (2004) Trends Cell Biol. **14**:443.