Recombinant Human Active ITK

Catalog Number: 4587-KS-010
Lot Number: 1732555

SPECIFICATIONS AND USE

Source	Recombinant human ITK (352-end) was expressed by baculovirus in *Sf9* cells using an N-terminal GST tag. The gene accession number is NM_005546.
Molecular Mass	The approximate molecular weight is 53 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of ITK was determined to be 35 nmol/min/mg using a Myelin Basic Protein (MBP) Substrate.
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.**

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).

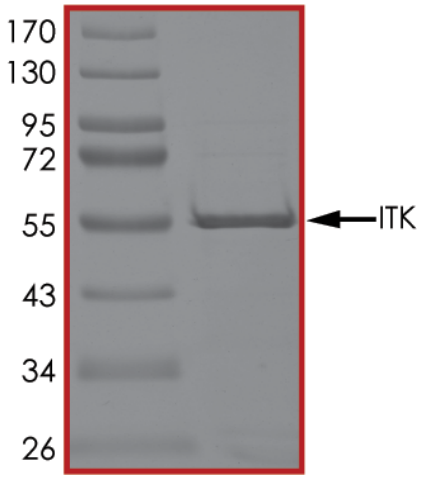

Figure 1: The purity of ITK was determined to be >90% by densitometry. Approximate MW 53kDa.

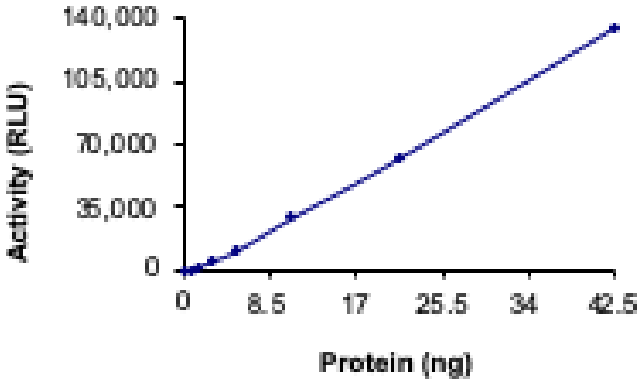

Figure 2: The specific activity of ITK was determined to be 35 nmol /min/mg as per Activity Assay Protocol, and was equivalent to 50 nmol/min/mg as per radiometric assay.

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.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active ITK (0.1 µg/µL) diluted with Kinase Dilution Buffer IX (1x) (and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$ and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1x) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - Myelin Basic Protein (MBP) diluted in 100 mM MOPS (pH 6.5) buffer to a final concentration of 0.5 mg/mL.

ASSAY PROCEDURE

The ITK assay is performed using the ADP-Glo™ Kinase Assay kit which quantifies the amount of ADP produced by the ITK reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active ITK, Kinase Assay Buffer III (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1X) in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5X)	79 µL
Substrate at 0.5 mg/mL	80 µL

3. Transfer the following reaction components prepared in step 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted the Active ITK
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see step 4) for each sample and calculate the Kinase Specific Activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)