ITK (Human), Active

Recombinant protein expressed in Sf9 cells

Cat# CY-SPI13

Lot No. P167-1
5 µg 0.1 µg/µl

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, 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).

Product Description:

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.

Gene Aliases:

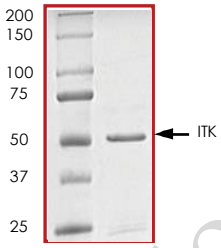
EMT; LYK; PSCTK2; MGC126257; MGC126258

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.

Purity & Molecular Weight:

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



Storage:

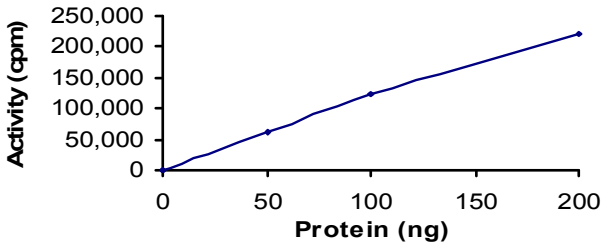
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at –70 °C from date of shipment.

Specific Activity:

The specific activity was determined to be 58 nmol /min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

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

5 X Kinase Assay Buffer:

25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

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.

CycLex Co., Ltd
1063-103 Tera-Sawaoka, Ina, Nagano, Japan 396-0002
Fax: 81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp