TEC, Active
Full-length recombinant human protein expressed in Sf9 cells

Catalog # T03-10G
Lot # N223-2

Product Description

Full-length recombinant human TEC was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The TEC gene accession number is NM_003215.

Gene Aliases

PSCTK4, MGC126760, MGC126762

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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

TEC is a member of the Tec family of non-receptor protein-tyrosine kinases that are involved in the intracellular signaling mechanisms of cytokine receptors, lymphocyte surface antigens, heterotrimeric G-protein coupled receptors, and integrin molecules. TEC is an integral component of T cell signaling and has a distinct role in T cell activation. Defects in TEC may be associated with myelodysplastic syndrome. TEC plays a crucial role in regulating FGF2 secretion under various physiological conditions (1) and it inhibits CD25 expression in human T-lymphocyte (2).

References


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**Activity Assay Protocol**

**Reaction Components**

**Active Kinase (Catalog #: T03-10G)**

Active TEC (0.1 µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 TEC for optimal results).

**Kinase Dilution Buffer IV (Catalog #: K24-09)**

KInase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

**Kinase Assay Buffer II (Catalog #: K02-09)**

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[33P]-ATP Assay Cocktail**

Prepare 250µM [33P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [33P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). 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 II (Catalog #: K02-09). Store 200µl aliquots at −20°C.

**Substrate (Catalog #: P61-58)**

Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

**Calculation of [P³³]-ATP Specific Activity (SA) (cpm/pmol)**

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*{Reaction time in min}*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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