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

Catalog # T03-10G
Lot # L2207-7

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
PSCK4, 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

Specific Activity
The specific activity of TEC was determined to be 9.5 nmol/min/mg as per activity assay protocol.

Purity
The purity of TEC was determined to be >70% by densitometry, approx. MW 103kDa.

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

Catalog # Specific Activity
T03-10G 9.5 nmol/min/mg
Lot # Purity Concentration Stability Storage & Shipping
L2207-7 >70% 0.05 µg/µl
1yr at −70°C from date of shipment
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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.
Activity Assay Protocol

**Reaction Components**

**Active Kinase** (Catalog #: T03-10G)
Active TEC (0.05 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.

**Assay Protocol**

Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active TEC, Kinase Assay Buffer, Substrate and Kinase 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 TEC (Catalog #T03-10G)
   - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #P61-58)
   - Component 3. 5µl of distilled H₂O

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 H₂O.

Step 5. Initiate the reaction by the addition of 5 µl [³³P]-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 20 µl 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 H₂O) 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 [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)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com

www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.