TXK, Active
Recombinant protein expressed in Sf9 cells
Catalog # T19-11G
Lot # P012-1

Product Description
Recombinant human TXK (239-end) was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is NM_003328.

Gene Aliases
RLK; TKL; BTKL; PTK4; PSCTK5; MGC22473

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.

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
TXK or RLK is a member of the TEC family of non-receptor tyrosine kinases. TXK is expressed in T-cells and is an important component of signaling pathways downstream of lymphocyte antigen receptor. TXK is phosphorylated in response to T-cell receptor stimulation and can be activated by phosphorylation by Src family kinases. However, TXK is phosphorylated independent of PI-3K activity (1). Excessive TXK protein expression is seen in patients with Behcet’s disease. Over production of TXK leads to increased Th1 cell function that is involved in the pathogenesis of Behcet’s disease (2).

References

Specific Activity
The specific activity of TXK was determined to be 18 nmol/min/mg as per activity assay protocol.

Purity
The purity was determined to be >90% by densitometry. Camk2α. Approx. MW ~53kDa.

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**Activity Assay Protocol**

**Reaction Components**

**Active Kinase (Catalog #: T19-11G)**
Active TXK (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 TXK for optimal results).

**Kinase Dilution Buffer III (Catalog #: K23-09)**
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/μl BSA solution.

**Kinase Assay Buffer I (Catalog #: K01-09)**
Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgC1₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[32P]-ATP Assay Cocktail**
Prepare 250μM [32P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [32P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). 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 I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

**Substrate (Catalog #: M42-54G)**
Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 0.2mg/ml.

**Assay Protocol**

**Step 1.** Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

**Step 2.** Thaw the Active TXK, 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 TXK (Catalog #T19-11G)
- Component 2. 10μl of 0.2mg/ml stock solution of substrate (Catalog #M42-54G)

**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 5μl [32P]-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 20μl 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 [P³²]–ATP Specific Activity (SA) (cpm/pmol)**
Specific activity (SA) = cpm for 5μl [³²P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

**Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)**
Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*[Reaction time in min]*[Enzyme amount in μg or mg])]*[Reaction Volume] / [Spot Volume]