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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
T04-11G-05	5 µg
T04-11G-10	10 µg
T04-11G-20	20 µg

TIE 2, Active

Recombinant protein expressed in Sf9 cells

Catalog # T04-11G
Lot # P287-1

Product Description

Recombinant human TIE 2 (771-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_000459.

Gene Aliases

TEK, VMCM, VMCM1, CD202B

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.

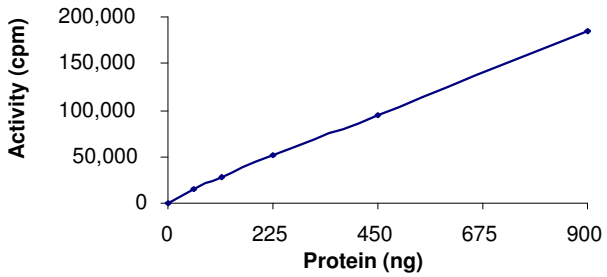
Scientific Background

TIE 2 or TEK is a receptor tyrosine kinase that is expressed principally on vascular endothelium. Disrupting TIE 2 function in mice results in embryonic lethality with defects in embryonic vasculature, suggesting a role in blood vessel maturation and maintenance. Angiopoietin-1 is a secreted growth factor that binds to and activates the TIE 2 receptor tyrosine kinase (1). SHP2 and GRB2 are recruited to the activated TIE 2 kinase domain and are part of the cellular responses that mediate TIE 2 function. TIE 2 expression is upregulated in the endothelium of vascular "hot spots" in human breast cancer specimens. However, TIE 2 is also overexpressed in areas of active angiogenesis in normal tissues (2).

References

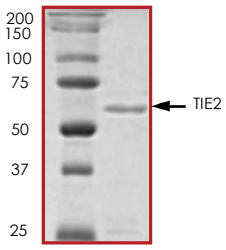
1. Woolf, A S. et al: Angiopoietin growth factors and Tie receptor tyrosine kinases in renal vascular development. Pediatr Nephrol. 2001 Feb;16(2):177-84.
2. Peters, K G. et al: Functional significance of Tie2 signaling in the adult vasculature. Recent Prog Horm Res. 2004;59:51-71.

Specific Activity



The specific activity of TIE 2 was determined to be **12 nmol /min/mg** as per activity assay protocol.

Purity



The purity of TIE 2 was determined to be **>90%** by densitometry. Approx. MW **61kDa**.

TIE 2, Active

Recombinant protein expressed in Sf9 cells

Catalog Number	T04-11G
Specific Activity	12 nmol/min/mg
Specific Lot Number	P287-1
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: T04-11G)

Active TIE 2 (0.1μg/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 TIE 2 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 50 ng/μl BSA solution.

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

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 12.5mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{32}P]-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

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H_2O to a final concentration of 1 mg/ml.

Assay Protocol

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

Step 2. Thaw the Active TIE 2, 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 TIE 2 (Catalog #T04-11G)
- **Component 2.** 10μl of 1 mg/ml stock solution of substrate.

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 H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{32}P]-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 H_2O) 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 [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{32}P]-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 ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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