

## Product: TIE 2, Active

Catalog #: 02-2083

Amount: 5 µg

### Product Description

Recombinant human TIE 2 (771-end) was expressed 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, 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.

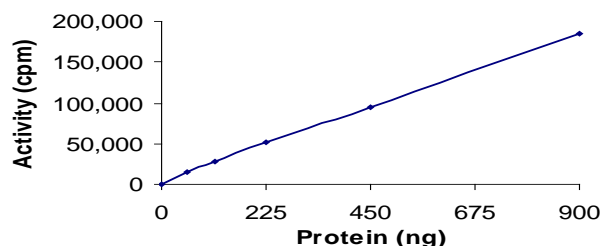
### Scientific Background

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

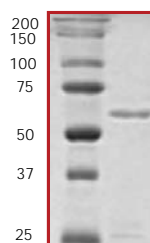
1. Woolf, A S. et al: Angiopoietin growth factors and Tie receptor tyrosine kinases in renal vascular development. *Pediatr Nephrol*. 2001 Feb;16(2):177-84.
2. Peters, K G. et al: Functional significance of Tie2 signaling in the adult vasculature. *Recent Prog Horm Res*. 2004;59:51-71.

### Specific Activity



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

### Purity



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

## TIE 2, Active

Recombinant protein expressed in Sf9 cells

Catalog Number **02-2083**  
 Quantity **5µg**  
 Specific Activity **12 nmol/min/mg**  
 Specific Lot Number **P287-1**

Purity **>90%**  
 Format **5µg in 50µl**  
 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 #: 02-2083)

Active TIE 2 (0.1µg/µl) diluted with Kinase Dilution Buffer (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, pH 7.2 (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, pH 7.2 (Catalog #: K02-09)

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

#### [γ]-ATP Assay Cocktail

Prepare 250µM [γ]-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 (Catalog #: K02-09). Store 1ml aliquots at –.

#### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K02-09). Store 200µl aliquots at –.

#### Substrate

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

### Assay Protocol

- Step 1. Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active TIE 2, Kinase Assay Buffer, Substrate and Enzyme 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 # 02-2083)
  - 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<sub>2</sub>O.
- 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 H<sub>2</sub>O) 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<sup>32</sup>]-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)]

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**MATERIAL SAFETY DATA SHEET****Article 1 - Product Identification and Use****Product Name: TIE 2, Active****Catalog # T04-11G-20**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: SignalChem Pharmaceuticals Inc.  
Street Address: 570-5600 Parkwood Way  
City, Prov. Postal Code: Richmond, BC, V6V 2M2  
Fax: 604-232-4601  
EMERGENCY PHONE: 604-232-4600

**Article 2 - Hazardous Ingredients**

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

**Article 3 - Physical Data**

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

**Article 4 - Fire and Explosion Hazard**

NOT APPLICABLE

**Article 5 - Reactivity Data**

NOT APPLICABLE

**Article 6 – Toxicologically Data**

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

**Article 7 - Preventative Measures**

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

\*\*\*\*\*MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES\*\*\*\*\*

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

**Article 8 - First Aid Measures**

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

**Article 9 - Preparation**

Prepared By:

Phone #:

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