

## TIE2 (K855A), Unactive

Recombinant human protein expressed in Sf9 cells

### Catalog # T04-16BG

Lot # C2007-5

### Product Description

Recombinant human kinase-dead TIE2 (K855A) (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

TIE-2, 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.

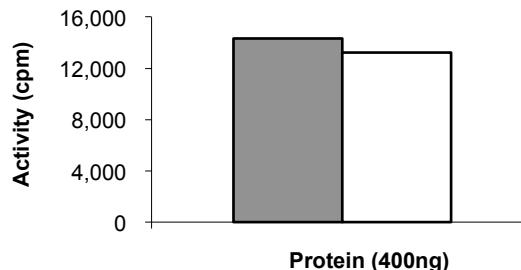
### Scientific Background

TIE2 or TEK is a receptor tyrosine kinase that is expressed principally on vascular endothelium. Disrupting TIE2 function in mice results in embryonic lethality with defects in embryonic vasculature, suggests a role in blood vessel maturation and maintenance. Angiopoietin-1 is a secreted growth factor that binds to and activates the TIE2 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 TIE2 function. TIE2 expression is upregulated in the endothelium of vascular "hot spots" in human breast cancer specimens. However, TIE2 is also overexpressed in areas of active angiogenesis in normal tissues (2).

### References

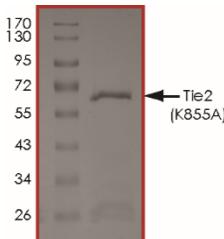
- Woolf, A S. et al: Angiopoietin growth factors and Tie receptor tyrosine kinases in renal vascular development. *Pediatr Nephrol*. 2001 Feb;16(2):177-84.
- Peters, K G. et al: Functional significance of Tie2 signaling in the adult vasculature. *Recent Prog Horm Res*. 2004;59:51-71.

### Specific Activity



Kinase-dead Tie2 (K855A) activity assay with or without substrate Poly (Glu:Tyr, 4:1) peptide. This recombinant Kinase-dead Tie2 mutant did not show significant kinase activity *in vitro* using the activity assay protocol in the data sheet.

### Purity



The purity of TIE2 (K855A) was determined to be >85% by densitometry.  
Approx. MW **65kDa**.

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Recombinant human protein expressed in Sf9 cells

Catalog #	T04-16BG
Specific Activity	32 nmol/min/mg
Lot #	C2007-5
Purity	>85%
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

### Unactive Kinase (Catalog #: T04-16BG)

Inactive TIE2 (K855A) (0.1 $\mu$ g/ $\mu$ l) 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 Inactive TIE2 (K855A) 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/ $\mu$ l BSA solution.

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

Buffer components: 25mM MOPS pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 20mM MgCl<sub>2</sub>, 12.5mM MnCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250 $\mu$ M [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 $\mu$ l [<sup>33</sup>P]-ATP (1mCi/100 $\mu$ l), 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 200 $\mu$ l aliquots at -20°C.

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

Poly (4:1 Glu, Tyr) peptide substrate diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Inactive TIE2 (K855A), 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 20 $\mu$ l:
  - Component 1.** 10 $\mu$ l of diluted Inactive TIE2 (K855A) (Catalog #T04-16BG)
  - Component 2.** 5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog # P61-58)
  - Component 3.** 5 $\mu$ l distilled H<sub>2</sub>O (4°C)
- 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 5 $\mu$ l [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l 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 $\mu$ l 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 $\mu$ l [<sup>33</sup>P]-ATP / pmoles of ATP (in 5 $\mu$ l of a 250 $\mu$ M ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]

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