

TIE2 (Human), Active

Recombinant protein expressed in Sf9 cells

Cat# CY-SPT04

Lot No. P287-1
5 µg 0.1 µg/µl

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).

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:

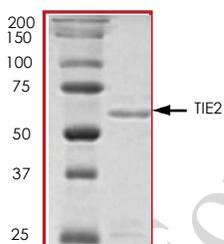
TEK, VMCM, VMCM1, CD202B

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

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



Storage:

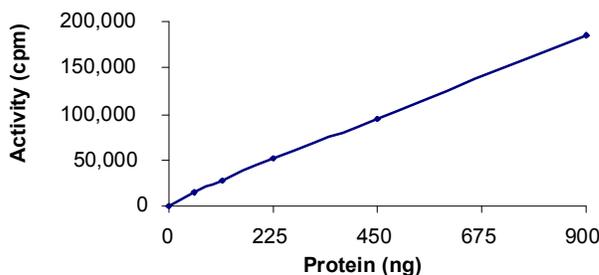
Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at -70°C from date of shipment.

**Specific Activity:**

The specific activity was determined to be 12 nmol /min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25 μ L reaction consisting of 5 μ L of 5 X Kinase Assay Buffer, 10 μ L of 1 mg/ml the Substrate Solution, 5 μ L of diluted kinase and 5 μ L of 250 μ M ATP solution containing [γ - 32 P] ATP (0.167 μ Ci/ μ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 μ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

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.

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.

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