KDR (Human), Active

Recombinant protein expressed in Sf9 cells

Cat# CY-SPK01

Lot No. 3
5 µg 0.1 µg/µl

Background:

KDR (or kinase insert domain receptor) is a growth factor receptor tyrosine kinase that was originally isolated from human endothelial cells where it plays a pivotal role in endothelial cell proliferation and differentiation. KDR and its mouse homolog Flk1 bind VEGF with high affinity and are implicated in the development of new blood vessels (angiogenesis) (1). The expression levels of VEGF and KDR are highly correlated during the normal development of the ocular vasculature in humans (1). Induction of angiogenesis is a critical step in tumor progression, and inhibitors of KDR have been demonstrated both to induce tumor regression and reduce metastatic potential in preclinical models (2).

Product Description:

Recombinant human KDR (789-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002253.

Gene Aliases:

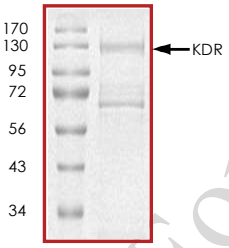
FLK1; VEGFR; VEGFR2

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 >70% by densitometry. Approx. MW 110kDa.



Storage:

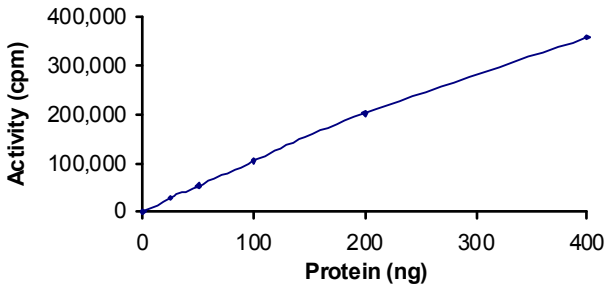
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 58 nmol /min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 5 µL of 1 mg/ml the Substrate Solution, 10 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL 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:

Myelin Basic Protein (MBP) substrate diluted in distilled H_2O to a final concentration of 1mg/ml.

5 X Kinase Assay Buffer:

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

References:

1.Neufeld, G. et al: Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999 Jan;13(1):9-22.

2.Zhu, Z. et al: Inhibition of tumor growth and metastasis by targeting tumor-associated angiogenesis with antagonists to the receptors of vascular endothelial growth factor. Invest New Drugs. 1999;17(3):195-212.

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