Recombinant Human VEGFR2/KDR/Flk-1 kinase domain His-tag

Catalog Number: 11740-KD

DESCRIPTION

Source

Spodoptera frugiperda, *Sf* 21 (baculovirus)-derived human VEGFR2/KDR/Flk-1 protein

Met	6-His tag	Sumo-tag (mutated, uncleavable)	3c Protease site	Human VEGFR2 (Ala792-Val1356) Accession # P35968.2

N-terminal Sequence Analysis: Protein identity confirmed by mass spectrometry.

Predicted Molecular Mass: 76 kDa

SPECIFICATIONS

SDS-PAGE: 87-101 kDa, under reducing conditions

Activity: Measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a synthetic peptide substrate.

The specific activity is >225 pmol/min/µg, as measured under the described conditions.

Endotoxin Level: <0.10 EU per 1 µg of the protein by the LAL method.

Purity: >85%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation: Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/mL BSA, pH 7.5
- Recombinant Human VEGFR2/KDR/Flk-1 kinase domain His-tag (rhVEGFR2) (Catalog # 11740-KD)
- Poly (4:1 Glu:Tyr), 1 mg/mL stock in 25 mM Tris, pH 7.5
- Adenosine triphosphate (ATP), 10 mM stock in deionized water
- ADP-Glo™ Kinase Assay (Promega)
- White 96-well Plate
- Plate Reader with Luminescence Read Capability

Assay

1. Dilute rhVEGFR2 to 1.6 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM ATP and 0.4 mg/mL Poly (4:1 Glu:Tyr) peptide in Assay Buffer.
3. Combine equal volumes of 1.6 ng/µL rhVEGFR2 and Substrate Mixture. Create a Substrate Control by replacing enzyme with Assay Buffer.
4. Incubate at room temperature for 40 minutes in the dark.
5. After incubation, transfer 10 µL of each reaction to wells of a white plate.
6. Terminate the reaction and deplete the remaining ATP by adding 10 µL of ADP-Glo Reagent (supplied in kit) to all wells.
7. Incubate at room temperature for 40 minutes in the dark.
8. Add 20 µL Kinase Detection Reagent (supplied in kit) to all wells.
9. Incubate at room temperature for 30 minutes in the dark.
10. Read plate in Luminescence endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Luminescence* (RLU) x Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Control

**Derived from ADP-Glo™ Kinase Assay Kit protocol (Promega)

Final Assay Conditions

Per Reaction:

- rhVEGFR2: 0.8 µg/mL
- ATP: 100 µM
- Poly (4:1 Glu:Tyr) peptide: 0.2 mg/mL

PREPARATION AND STORAGE

Shipping: The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage: **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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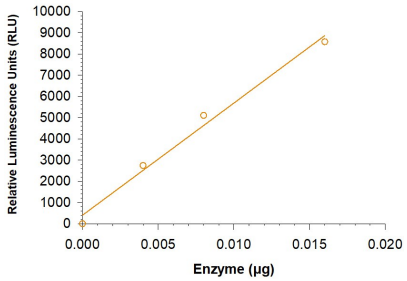
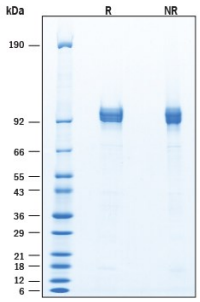
Enzyme Activity



Recombinant Human VEGFR2/KDR/Flk-1 kinase domain His-tag Enzyme Activity. Recombinant Human VEGFR2/KDR/Flk-1 kinase domain His-tag (Catalog # 11740-KD) is measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a synthetic peptide substrate.

SDS-PAGE



Recombinant Human VEGFR2/KDR/Flk-1 kinase domain His-tag SDS-PAGE. 2 µg/lane of Recombinant Human VEGFR2/KDR/Flk-1 kinase domain His-tag (Catalog # 11740-KD) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 87-101 kDa, under reducing conditions.

BACKGROUND

Vascular endothelial growth factor receptor 2 (VEGFR2), also known as Fetal liver kinase 1 (FLK-1) and Kinase insert domain receptor (KDR), is one of three ubiquitously expressed, highly conserved tyrosine protein kinase VEGFRs from the platelet-derived growth factor receptor (PDGFR) family (1). VEGFR2, like other receptor members in this family is comprised of an extracellular domain (ECD) with immunoglobulin-like segments that binds its target cytokine VEGF to induce dimerization, a transmembrane domain, and a cytosolic kinase domain (1). Similar to the structure of other tyrosine kinases, the kinase domain contains the conserved catalytic domain with an ATP binding site within the cleft of two lobes at the N-terminal and C-terminal regions as well as the glycine-rich or nucleotide binding loop and activation loop (1,2). Unique to this family there is also a kinase insert domain (KID) of unknown function (1). Through ligand binding, dimerization, activation via autophosphorylation, and subsequent phosphorylation of targets in multiple signaling pathways, VEGFR2 regulates endothelial cell growth, migration, and survival and plays a critical role in angiogenesis (2-4). Abnormal angiogenesis is a hallmark of tumor progression and metastasis; VEGFR-2 has been reported to be overexpressed and overstimulated in tumors and thus closely linked to the occurrence and development of multiple types of tumors (2,5,6). Targeting inhibition of VEGFR2 is a promising cancer therapeutic strategy based on multiple clinical trials (2,4,7). There is significant research and interest in the development of drugs targeting VEGFR2 inhibition (8) especially including dual inhibition in combination with other kinases or other proteins (9-15).

References:

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