Flt1 N-Terminal His Tag. Sf9 cells Active Enzyme

EGFR-1, Vascular permeability factor receptor, Tyrosine-protein kinase receptor FLT, Tyrosine-protein kinase FRT, Fms-like tyrosine kinase 1

Background

Receptor for VEGF, VEGFB and PGF. Has a tyrosine-protein kinase activity. The VEGF-kinase ligand/receptor signaling system plays a key role in vascular development and regulation of vascular permeability. Isoform SFlt1 may have an inhibitory role in angiogenesis.

Description: Human recombinant Flt1 (FMS-like tyrosine kinase), also known as VEGFR1 (Vascular endothelial growth factor receptor 1), GenBank Accession No. AF063657, a.a. 783-end with N-terminal His tag, MW=67.5 kDa, expressed in a Baculovirus infected Sf9 cell expression system.

Activity

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling. Specific Activity: 170 U/mg. One unit is defined as the amount of enzyme that will transfer 1 nmol phosphate to the tyrosine substrate per minute at pH 7.4 and 30ºC. Assay buffer: 50 mM HEPES, pH 7.4, 3 mM MgCl2, 3 mM MnCl2, 1 mM DTT, 3 uM Na-orthovanadate,

Purity

>70% by SDS-PAGE

Applications

Ordering Information

Catalog Number
X1758E

Size
10 µg

Customer Storage
Product should be stored at -80ºC. Aliquot to avoid freeze/thaw cycles

Formulation
25 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.05% Tween-20, 50% glycerol, and 3 mM DTT.

Ship Conditions
Ship on dry ice, freeze upon arrival

Stability
Stable for > 6 months when stored as recommended.

Concentration
170 units/µg

Source
Baculovirus infected Sf9 cells

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ASSAY METHODS

MATERIALS

Use Invitrogen Z'-Lyte kinase assay kit - Tyrosine 4 peptide

PROCEDURE

1. Make 1X assay buffer.

2. Make substrate solution (1:250 dilution of the stock solution in 1X assay buffer containing 400 μM ATP). The final concentration is 2 μM for Tyr4 peptide substrate and 200 μM for ATP.

3. Make phosphorylated Tyr 4 peptide substrate solution (1:250 dilution of the stock in 1X assay buffer). The final concentration of phosphorylated Tyr 4 peptide substrate is 2 μM

4. Dilute FLT1 to 20 ng /μl with 1X assay buffer, and transfer 25 μl to each well (500 ng FLT1 per reaction). Add 25 μl of 1X assay buffer to the designated well for a negative control and the another designated well for a positive control.

5. Add 25 μl of substrate solution (from step 2) to wells containing Flt1 and the negative control, and 25 μl of phosphorylated Tyr4 peptide substrate (from step 3) to the designated well for the positive control.

6. Incubate the reaction at room temperature for 1 hour.

7. Make a developer solution by diluting developer reagent B in developer dilution buffer (1:32 dilution).

8. Add 25 μl of diluted developer to each well.

9. Incubate the reaction at room temperature for 1 hour.

10. Add 25 μl of Stop solution to each well.

11. Read fluorescence intensity at exc360/em460 and

REFERENCES

1. Rahimi, N., et al. J. Biol. Chem., 275: 16986-16992, 2000

2. Shibuya, M. Int. J. Biochem. Cell Biol., 33: 409-420, 2001

PRODUCT SPECIFIC REFERENCES

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