Flt3 N-Terminal HIS Tag. Sf9 cells Active Enzyme

Fms-related tyrosine kinase 3 ligand, Flt3 ligand, Flt3L

Background

Stimulates the proliferation of early hematopoietic cells. Synergizes well with a number of other colony stimulating factors and interleukins.

Description: Human Flt3 (GenBank Accession No. NM_004119), (a.a. 564-end) with N-terminal His tag, MW=54.2 kDa, expressed in a Baculovirus infected Sf9 cell expression system.

Activity

Specific Activity: 225 pmol/min/µg. Assay condition: The enzyme reactionwas carried out for 1h at room temperature in a buffer containing 50 mM HEPES (pH7.5), 10 mM MgCl2, 1 mM EDTA, 0.01% BRIJ35 and 200 µM of ATP. Substrate: 2 µM Tyr peptide 2 from Invitrogen. Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

Purity

>80%

Applications

Ordering Information

Catalog Number
X1759E

Size
10 µg

Customer Storage
Product should be stored at -80°C. 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

Stability
Stable for > 6 months when stored as recommended.

Concentration
See vial for concentration

Source
Human Flt3 (GenBank Accession No. NM_004119), (a.a. 564-end) with Nterminal His tag, MW=54.2 kDa, expressed in a Baculovirus infected Sf9 cell expression system.

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ASSAY METHODS

MATERIALS

Use Invitrogen Z'-Lyte kinase assay kit - Tyrosine 2 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 Tyr2 peptide substrate and 200 μM for ATP.

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

4. Dilute FLT3 to 0.2 ng /ul with 1X assay buffer, and transfer 25 μl to each well (5ng FLT3 per reaction). Add 25μl of 1X assay buffer to empty wells for a negative control and a positive control.

5. Add 25 μl substrate solution (from step 2) to the wells containing Flt3 and the designated negative control, and 25 ul of phosphorylated Tyr 2 peptide substrate (from step 3) to the designated positive well.

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

7. Make a developer solution by diluting developer reagent A in developer dilution buffer (1:128 dilution).

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

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

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

11. Read fluorescence intensity at exc360/em460 and

REFERENCES

1. Takahashi,S., et al., Leuk. Res. 29 (8), 893899 (2005)

2. Levis,M., et al., Blood 106 (2), 673-680 (2005).

PRODUCT SPECIFIC REFERENCES

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