Recombinant Mouse HGF Activator

Catalog Number: 1200-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Met1-Ser653 with a C-terminal 10-His tag Accession # Q9R098 The pro form was purified, activated and further purified.
N-terminal Sequence Analysis	Val370 & Ile406
Structure / Form	Active form
Predicted Molecular Mass	32 kDa & 28 kDa

SPECIFICATIONS

SDS-PAGE	35 kDa and 32 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The specific activity is >100 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.01% CHAPS, pH 9.0
- Recombinant Mouse HGFA (rmHGFA) (Catalog # 1200-SE)
- Substrate MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH_2 (Catalog # ES002) , 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmHGFA to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 50 µL of 2 ng/µL rmHGFA in a plate, and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank by combining 50 µL of 20 µM Substrate with 50 µL of Assay Buffer.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rmHGFA: 0.100 µg]
- Substrate: 10 µM

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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Hepatocyte Growth Factor Activator (HGFA) is a serine endopeptidase that cleaves at the peptide bond between Arg494 and Val495 of single-chain human HGF precursor, generating the active heterodimer (1). HGFA is produced and secreted by the liver and normally circulates in the blood as an inactive zymogen (2, 3). The zymogen has a weak affinity for heparin but acquires a strong affinity for heparin upon activation that is linked to blood coagulation. This property may ensure the local action of this enzyme at the site of tissue injury (3). Mouse HGFA precursor (653 amino acid residues) contains several predicted domains including a signal peptide (residues 1-29), a propeptide (residues 30-369), and a mature and active form (residues 370 to 653) that is further processed into a short chain (residues 370-405) and a long chain (residues 406-653). The short chain and the long chain (catalytic domain) may form a disulfide bond linked dimer. HGFA can be activated by autocatalysis or by thrombin (4). The active protease can be inhibited by HGFA inhibitors (HAIs). Two HAIs, HAI-1 and HAI-2, are known in mouse and human. HAI-1 is not only an inhibitor, but also a specific acceptor of active HGFA, acting as a reservoir of this enzyme on the cell surface (5).

References:

1. Kitamura, N. (2004) in *Handbook of Proteolytic Enzymes* (Barrett, A.J. *et al.* Eds.), p. 1712, Academic Press, San Diego.
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