Recombinant Human HAI-1
Catalog Number: 1048-PI

DESCRIPTION

Source
Mouse myeloma cell line, NS0-derived
Pro37-Glu449, with a C-terminal 10-His tag
Accession # NP_003701

N-terminal Sequence Analysis
Pro37

Predicted Molecular Mass
47 kDa

SPECIFICATIONS

SDS-PAGE
58 kDa, reducing conditions

Activity
Measured by its ability to inhibit trypsin cleavage of a fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH2 (Catalog # ES002). The IC50 is <2 nM, 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
Lyophilized from a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human HAI-1 (rhHAI-1) (Catalog # 1048-PI)
- Trypsin (Sigma, Catalog # T-1426)
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-Val-Arg-Lys(DNP)-NH2 (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute Trypsin to 0.25 μg/mL in Assay Buffer.
2. Prepare a curve of rhHAI-1 (MW: 47489 Da) in Assay Buffer. Make the following serial dilutions: 500, 200, 50, 25, 10, 5, 2, 0.5, and 0.05 nM.
3. Mix equal volumes of the rhHAI-1 curve dilutions and the diluted Trypsin. Include a control (in duplicate) containing Assay Buffer and the diluted Trypsin.
4. Incubate reactions for 1 hour at 37 °C.
   After incubation, dilute the mixtures 5 fold in Assay Buffer.
5. Dilute Substrate to 20 μM in Assay Buffer.
6. Load 50 μL of the diluted incubated mixtures into a plate and start the reaction by adding 50 μL of 20 μM Substrate.
7. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
8. Derive the 50% inhibiting concentration (IC50) of rhHAI-1 by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
9. The specific activity for trypsin at each point may be determined using the following formula (if needed):

   Specific Activity (pmol/min/μg) = \[
   \frac{Adjusted \ V_{max} \times (RFU/min) \times Conversion \ Factor}{amount \ of \ enzyme (μg)}
   \]

   *Adjusted for Substrate Blank

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

Final Assay Conditions
Per Well:
- Trypsin: 0.00125 μg
- rhHAI-1 curve: 25, 10, 2.5, 1.25, 0.5, 0.25, 0.1, 0.025, 0.0025 nM
- Substrate: 10 μM

PREPARATION AND STORAGE

Reconstitution
Reconstitute at 100 μg/mL in sterile, deionized water.

Shipping
The product is shipped with polar packs. 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 reconstitution.

Rev. 2/6/2018 Page 1 of 2

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HAI-1 is a Kunitz-type serine protease inhibitor, identified as a strong inhibitor of HGF activator (HGFA) and matriptase (1). The membrane-anchored HAI-1 consists of two Kunitz domains, a LDL-receptor-like domain, and a C-terminal transmembrane domain (2). Two soluble forms are generated by ectodomain shedding, one with a single Kunitz domain and the other with two Kunitz domains. HAI-1 is not only an inhibitor but also a specific receptor of active HGFA, acting as a reservoir of this enzyme on the cell surface (3). The shedding of HAI-1 and HGFA/HAI-1 complex is enhanced by treatment with phorbol 12-myristate 13-acetate or IL-1β. The regulated shedding is completely inhibited by a synthetic zinc metalloprotease inhibitor (3).

References: