Recombinant Human HAI-2A

Catalog Number: 1106-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Met1-Lys197, with a C-terminal 10-His tag Accession # O43291
N-terminal Sequence Analysis	Ala28
Predicted Molecular Mass	21 kDa

SPECIFICATIONS

SDS-PAGE	27 kDa and 32 kDa, reducing conditions
Activity	Measured by its ability to inhibit trypsin cleavage of a fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The IC_{50} value is <4.6 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, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human HAI-2A (rhHAI-2A) (Catalog # 1106-PI)
- Trypsin (Sigma, Catalog # T-1426)
- 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 Trypsin to 0.25 µg/mL with Assay Buffer.
2. Prepare a curve of rhHAI-2A (MW: 20539 Da) in Assay Buffer. Make the following serial dilutions: 1000, 100, 33.3, 16.7, 8.33, 4.17, 2.08, and 0.417 nM.
3. Mix equal volumes of the rhHAI-2A curve dilutions and the diluted Trypsin. Include a control (in duplicate) containing Assay Buffer and the diluted Trypsin without any rhHAI-2A.
4. Incubate reactions for 30 minutes at room temperature.
5. After incubation, dilute the mixtures by 5 fold in Assay Buffer.
6. Dilute Substrate to 20 µM in Assay Buffer.
7. Load 50 µL of the diluted incubated mixtures into a plate, and start the reaction by adding 50 µL of 20 µM Substrate.
8. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
9. Derive the 50% inhibition concentration (IC_{50}) by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
10. The specific activity for Trypsin at each point may be determined using the following formula (if needed):

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- Trypsin: 0.00125 µg
- rhHAI-2A curve: 50, 5, 1.67, 0.83, 0.417, 0.209, 0.104, and 0.0209 nM
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile, deionized water.
Shipping	The product is shipped at ambient temperature. 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.

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BACKGROUND

Two alternatively spliced forms of HAI-2 have been found in human tissues (1). HAI-2A, the full-length molecule and also known as placental bikunin, is a major form expressed in human tissues. Encoded by the SPINT2 gene, HAI-2A consists of two Kunitz domains, and a C-terminal transmembrane domain (1-5). Both Kunitz domains can function as inhibitors independent of each other. In addition to HGF activator and trysin, HAI-2A strongly inhibits plasmin, tissue and plasma kallikreins, and factor XIa. In comparison, HAI-2A is a weaker inhibitor of factor VIIa-tissue factor, factors IXa, Xa, and XIIa. Recombinant HAI-2A prolonged the clotting time in an activated partial thromboplastin time assay.

References:

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