Recombinant Human Hepsin

Catalog Number: 4776-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Arg45-Leu417 (Asp161Glu & Arg162Lys), with a C-terminal 10-His tag Accession # P05981
N-terminal Sequence Analysis	Arg45
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	40-43 kDa, reducing conditions
Activity	Measured by its ability to cleave *tert*-butoxycarbonyl-Gln-Arg-Arg-7-amino-4-methylcoumarin (Boc-QRR-AMC). The specific activity is >20,000 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 Sodium Acetate and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 0.1 M Tris, 10 mM $CaCl_2$, 0.15 M NaCl, 0.05 % Brij-35, pH 8.0
- Assay Buffer: 50 mM Tris, pH 9.0
- Recombinant Human Hepsin (rhHepsin) (Catalog # 4776-SE)
- Fluorogenic Peptide Substrate: BOC-Gln-Arg-Arg-AMC (Bachem, Catalog # I-1655), 5 mM stock in 50:50 DMSO:Methanol
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhHepsin to 100 µg/mL in Activation Buffer.
2. Incubate at 37 °C for 24 hours.
3. Dilute activated rhHepsin to 0.2 ng/µL in Assay Buffer.
4. Dilute Substrate to 200 µM in Assay Buffer.
5. Load 50 µL of the 0.2 ng/µL rhHepsin in a black well plate and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM Substrate.
6. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in kinetic mode for 5 minutes.
7. 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 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhHepsin: 0.010 µg
- Substrate: 100 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

Hepsin, also known as TMPRSS1, is a Type II membrane protein with an extracellular serine protease domain (1). It is most highly expressed in liver, but is also present in many other tissues, notably lung, kidney, and skeletal muscle (2). A soluble form of Hepsin lacking the transmembrane domain has been identified (3). Hepsin is capable of activating Factor VII, and may initiate blood coagulation at the cell surface (4). Hepsin is overexpressed in various human tumors, including prostate (5), and is considered to be a biomarker for prostate cancer (6). Recombinant human Hepsin was expressed as a secreted, soluble protein lacking its cytosolic and transmembrane domains. The D161E and R162K mutations were introduced into the prosequence to improve expression of the recombinant human Hepsin.

References:

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