Recombinant Human Airway Trypsin-like Protease/HAT

Catalog Number: 2695-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala42-Ile418 with an N-terminal 6-His tag Accession # O60235 The protein was purified, activated and further purified.
N-terminal Sequence Analysis	Ile187 & His
Structure / Form	Active
Predicted Molecular Mass	25 kDa & 17 kDa

SPECIFICATIONS

SDS-PAGE	27 kDa and 18 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, t-butoxycarbonyl-Phe-Ser-Arg-7-amino-4-methyl coumarin (Boc-FSR-AMC). The specific activity is >50,000 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	>90%, 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, 0.05% (w/v) Brij-35, pH 9.5
- Recombinant Human Airway Trypsin-like Protease/HAT (rhHAT) (Catalog # 2695-SE)
- Substrate: t-Butyloxycarbonyl Phe-Ser Arg 7-amino-4-methyl coumarin (Bachem, Catalog # I-1400), 10 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 rhHAT to 0.02 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. In a plate load 50 µL of 0.02 ng/µL rhHAT, and start the reaction by adding 50 µL of 200 µM Substrate to wells. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 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 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhHAT: 0.001 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM Tris, 150 mM NaCl and 0.05% Brij-35, pH 7.5.
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.

BACKGROUND

HAT was initially purified from the sputum of patients with chronic airway diseases (1). Subsequent cloning revealed it as a member type II transmembrane serine proteases (2, 3). HAT has been shown to induce PAR-2 mediated IL-8 release in psoriasis vulgaris and increase mucin expression in airway epithelial cells (4, 5). Located in the cells of the submucosal serous glands of the bronchi and trachea, the isolated enzyme had the N-terminal sequence of ILGGTEAEEG, which corresponded to the start of the C-terminal catalytic domain (residues 187 to 418) of the deduced sequence (1, 2). The N-terminal region consisted of a short cytoplasmic tail (residues 1 to 20), a transmembrane domain (residues 21 to 41), and a SEA domain (residues 44 to 164). The ectodomain of HAT is expressed and the active enzyme is purified.

References:

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2. Yamaoka, K. *et al.* (1998) J. Biol. Chem. **273**:11895.
3. Hooper, J.D. *et al.* (2001) J. Biol. Chem. **276**:857.
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5. Chokki, M. *et al.* (2004) Am. J. Respir. Cell Mol. Biol. **30**:470.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449