Recombinant Mouse Mast Cell Protease-11/Prss34

Catalog Number: 2857-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse Mast Cell Protease-11/Prss34 protein Met20-Ser318, with a C-terminal 10-His tag Accession # Q80UR4
N-terminal Sequence Analysis	Met20
Structure / Form	Pro form
Predicted Molecular Mass	34 kDa

SPECIFICATIONS

SDS-PAGE	48 kDa doublet, reducing conditions
Activity	Measured by its ability to cleave a colorimetric peptide substrate, N-carbobenzyloxy-Arg-ThioBenzyl ester (Z-R-SBzl), in the presence of 5,5'Dithio-bis (2-nitrobenzoic acid) (DTNB). Edwards, K.M. *et al.* (1999) J. Biol. Chem. **274**:30468. 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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 0.15 M NaCl, 10 mM $CaCl_2$, pH 7.5 (TCN)
- Assay Buffer: 50 mM Tris, pH 8.0
- Recombinant Mouse Mast Cell Protease-11/Prss34 (rmMCP-11) (Catalog # 2857-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: Z-Arg-SBzl (SM Biochemicals, Catalog # SMSB01), 10 mM in DMSO
- 5,5'Dithio-bis(2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D-8130)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rmMCP-11 to 200 µg/mL with Activation Buffer.
2. Dilute Thermolysin to 0.4 µg/mL with Activation Buffer.
3. Mix equal volumes of 200 µg/mL rmMCP-11 and 0.4 µg/mL Thermolysin for final concentrations of 50 µg/mL and 0.2 µg/mL, respectively.
4. Incubate at 37 °C for 30 minutes.
5. Stop the reaction with 10 mM 1,10 Phenanthroline.
6. Dilute activated rmMCP-11 to 0.1 ng/µL in Assay Buffer.
7. Dilute substrate to 200 µM in Assay Buffer with 200 µM of DTNB.
8. Load 50 µL of the 0.1 ng/µL rmMCP-11 into plate, and start the reaction by adding 50 µL of the substrate/DTNB mixture to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL substrate mix without any rmMCP-11.
9. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.
10. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rmMCP-11: 0.005 µg
- DTNB: 100 µM
- 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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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

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BACKGROUND

Mast Cell Protease-11 (MCP-11) is encoded by Prss34, one of 13 genes on mouse chromosome 17A3.3 that correspond to functional trypsin-like serine proteases (1). The deduced amino acid sequence of mouse MCP-11 consists of 318 residues with a signal peptide (residues 1 to 19), a pro region (residue 20 to 34), and a catalytic domain (35 to 318). The mRNA is preferentially expressed in spleen and bone marrow. The mouse MCP-11 (residues 20 to 318) was expressed in the NS0 cells with a foreign signal peptide. After being treated with thermolysin, the purified enzyme is active against a peptide substrate described in the Activity Assay Protocol. Apparently, the human gene corresponding to Prss34 encodes a protein that is not enzymatically active due to a mutation that leads to a premature translation termination codon.

References:

1. Wong, G.W. *et al.* (2004) J. Biol. Chem. **279**:2438.

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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