Recombinant Mouse Kallikrein 1

Catalog Number: 7928-SE

DESCRIPTION

Source

Mouse myeloma cell line, NS0-derived

Mouse Kallikrein 5 precursor (Val23-Arg67) Accession # NP_081082	Mouse Kallikrein 1 (Ile25-Asp261) Accession # P15947	6-His tag

N-terminus C-terminus

N-terminal Sequence Analysis

Val23 (Mouse Kallikrein 5 precursor)

Predicted Molecular Mass

32 kDa

SPECIFICATIONS

SDS-PAGE

40-45 kDa, reducing conditions

Activity

Measured by its ability to cleave a flourogenic peptide substrate Pro-Phe-Arg-7-amido-4-methylcoumarin (PFR-AMC).
The specific activity is >15,000 pmol/min/µg, as measured under the described conditions.

Endotoxin Level

<0.01 EU per 1 µg of the protein by the LAL method.

Purity

>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.

Formulation

Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, pH 7.5 (TCN)
- Assay Buffer: 50 mM CHES, 250 mM NaCl, pH 10.0
- Recombinant Mouse Kallikrein 1 (rmKLK1) (Catalog # 7928-SE)
- Thermolysin (Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: Pro-Phe-Arg-AMC (Bachem, Catalog # I-1295), 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 rmKLK1 to 200 µg/mL in Activation Buffer.
2. Dilute Thermolysin to 2 µg/mL in Activation Buffer.
3. Combine 20 µL of diluted rmKLK1 with 20 µL of diluted Thermolysin for final concentrations of 100 µg/mL and 1 µg/mL respectively.
4. Incubate at 37 °C for 1 hour.
5. Stop the reaction with 40 µL of 20 mM 1,10 Phenanthroline for a final concentration of 10 mM.
6. Dilute incubated rmKLK1 to 0.2 ng/µL in Assay Buffer.
7. Dilute Substrate to 200 µM in Assay Buffer.
8. Load 50 µL of the 0.2 ng/µL rmKLK1 in a 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.
9. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
10. 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:

- rmKLK1: 0.01 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

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 opening.

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BACKGROUND

The kallikreins are a family of trypsin-like serine proteases, many of which are associated with a variety of cancers (1). Kallikrein 1 (KLK1) is also known as tissue kallikrein and urinary kallikrein. An important physiological function of KLK1 is the cleavage of kininogen to release a vasoactive kinin peptide, bradykinin (for rodent KLK1) or lysyl-bradykinin (for human KLK1) (2, 3). Kinins regulate vasodilation, blood pressure reduction, smooth muscle relaxation and contraction, pain induction and inflammation. Recombinant mouse KLK1 was expressed with a human CD33 signal peptide and a mouse KLK5 pro-peptide, followed by the mouse KLK1 catalytic domain (residues 25 to 261). The recombinant mouse KLK1 was purified as the latent pro-form, which is readily activated by treatment with thermolysin.

References:

1. Avgeris, M. *et al.* (2012) Biol. Chem. **393**:301.
2. Hosoi, K. *et al.* (1994) J. Biochem. **115**:137.
3. Kato, H. *et al.* (1987) J. Biochem. **102**:1389.

PRODUCT SPECIFIC NOTICES

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