Recombinant Human Kallikrein 7

Catalog Number: 2624-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Glu23-His252, with a C-terminal 10-His tag Accession # P49862
N-terminal Sequence Analysis	Glu23
Structure / Form	Pro form
Predicted Molecular Mass	26 kDa

SPECIFICATIONS

SDS-PAGE	29 kDa and 32 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The specific activity is >150 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, 150 mM NaCl, pH 8.5
- Recombinant Human Kallikrein 7 (rhKLK7) (Catalog # 2624-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- EDTA (Sigma, Catalog # E-4884)
- Substrate: MCA-Arg-Pro Lys-Pro-Val-Glu-Nval-Trp-Arg-Lys(Dnp)-NH_2 (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhKLK7 to 200 µg/mL in Activation Buffer.
2. Dilute Thermolysin to 20 µg/mL in Activation Buffer.
3. Combine equal volumes of 200 µg/mL rhKLK7 and 20 µg/mL Thermolysin.
4. Incubate at 37 °C for 2 hours.
5. Stop reaction with an equal volume 100 mM EDTA diluted in Assay Buffer (C_f = 50 mM).
6. Dilute activated rhKLK7 to 4 ng/µL in Assay Buffer.
7. Dilute Substrate to 20 µM in Assay Buffer.
8. Load 50 µL of 4 ng/µL rhKLK7 in a plate, and start the reaction by adding 50 µL of 20 µM Substrate to each well. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate.
9. Read at excitation and emission wavelengths of 320 nm and 405 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 MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhKLK7: 0.2 µg
- Substrate: 10 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.

DATA

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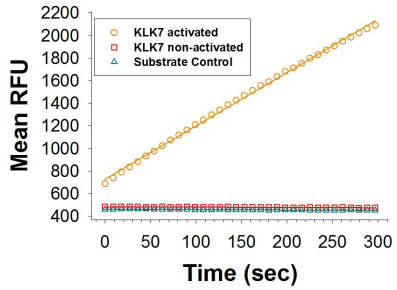
Enzyme Activity



Recombinant Human Kallikrein 7 (Catalog # 2624-SE) is measured by its ability to cleave the fluorogenic peptide substrate Mca-RPKPVE-Nval-WRK(Dnp)-NH2 (Catalog # ES002).

BACKGROUND

Human tissue Kallikrein 7 (hK7), also known as stratum corneum chymotryptic enzyme (SCCE), is a member of the human tissue kallikrein family. Full-length hK7 consists of 253 amino acids, with a signal peptide (residues 1-22), short pro peptide (residues 23-29) and mature chain (residues 30-252) (1). Predominantly expressed in the skin, a major physiological function of hK7 is to regulate the desquamation process through proteolysis of the intercellular adhesive structures between corneocytes (2). Thus, it is related to some inflammatory skin diseases, such as psoriasis and chronic itchy dermatitis (3, 4). Studies have shown that one potential physiological activator for hK7 is hK5, another member of the human tissue Kallikrein family. Along with hK14, these three kallikreins form a proteolytic cascade in the stratum corneum (5). The purified, secreted recombinant human K7 corresponds to the pro form. When activated by thermolysin, it displays enzymatic activity towards a fluorogenic synthetic peptide described in the Activity Assay Protocol. This activity can be inhibited by recombinant human Serpin A1, A3, A4, and A5 (Catalog # 1268-PI, 1295-PI, 1669-PI, and 1266-PI).

References:

1. Hansson, L. *et al.* (1994) J. Bio. Chem. **269**:19420.
2. Caubet, C. *et al.* (2004) J. Invest. Dermatol. **122**:1235.
3. Ekholm, E. and Egelrud, T. (1999) Arch. Dermatol. Res. **291**:195.
4. Hansson, L. *et al.* (2002) J. Invest. Dermatol. **118**:444.
5. Brattsand, M. *et al.* (2004) J. Invest. Dermatol. **124**:198.

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