DESCRIPTION

Source
Mouse myeloma cell line, NS0-derived
Gln22-Arg249 with a C-terminal 6-His tag
Accession # Q91VE3

N-terminal Sequence Analysis
No results obtained: Gln22 predicted

Predicted Molecular Mass
26 kDa

SPECIFICATIONS

SDS-PAGE
27-37 kDa, reducing conditions

Activity
Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKV-E-Nval-WRK(Dnp)-NH₂ (Catalog # ES002).
The specific activity is >70 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 Colloidal Coomassie® Blue stain at 5 μg per lane.

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, 150 mM NaCl, 10 mM CaCl₂, 0.05% Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, 1 M NaCl, pH 8.0
- Recombinant Mouse Kallikrein 7 (rmKLK7) (Catalog # 7688-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- EDTA, Disodium salt (Sigma, Catalog # E-4884) 500 mM stock in deionized water
- Substrate: Mca-RPKV-E-Nval-WRK(Dnp)-NH₂ (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmKLK7 to 200 μg/mL in Activation Buffer.
2. Dilute Thermolysin to 20 μg/mL in Activation Buffer.
3. Activate rmKLK7 by combining equal volumes of 200 μg/mL rmKLK7 with 20 μg/mL Thermolysin.
4. Incubate reactions at 37 °C for two hours.
5. Stop reactions by adding EDTA to a final concentration of 50 mM.
6. Dilute activated rmKLK7 to 2 μg/mL in Assay Buffer.
7. Dilute Substrate to 20 μM in Assay Buffer.
8. In a plate, load 50 μL of activated rmKLK7. Include an enzyme blank containing 50 μL Assay Buffer.
9. Start reaction by adding 50 μL of 20 μM Substrate.
10. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
11. Calculate specific activity:
    \[
    \text{Specific Activity (pmol/min/μg)} = \left( \frac{\text{Adjusted } V_{\text{max}} \cdot (\text{RFU/min}) \times \text{Conversion Factor}^* \ (\text{pmol}/RFU)}{\text{amount of enzyme (μg)}} \right)
    \]
    *Adjusted for Substrate Blank
    **Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rmKLK7: 0.1 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.
Kallikrein 7 (KLK7), also known as stratum corneum chymotryptic enzyme (SCCE), is a member of the tissue kallikrein family. Predominantly expressed in the skin, a major physiological function of KLK7 is to regulate the desquamation process through proteolysis of the intercellular adhesive structures between corneocytes (1). Thus, it is involved in some inflammatory skin diseases, such as psoriasis and chronic itchy dermatitis (2, 3). Studies have shown that one potential physiological activator for KLK7 is KLK5, another member of the tissue kallikrein family. Along with KLK14, these three kallikreins form a proteolytic cascade in the stratum corneum (4). Mouse KLK7 is synthesized as a 249 amino acid precursor with a 21 amino acid signal peptide. The secreted protein has a short propeptide (residues 22-25) and a mature chain comprised of residues 26-249. The purified, secreted rmKLK7 corresponds to the pro form. After activation by thermolysin, it displays enzymatic activity against a fluorogenic synthetic peptide as described in the Activity Assay Protocol.

References:

PRODUCT SPECIFIC NOTICES
Coomassie is a registered trademark of Imperial Chemical Industries Ltd.