Recombinant Human Kallikrein 4/Prostase/EMSP1

Catalog Number: 1719-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ser27-Ser254, with a C-terminal 10-His tag Accession # NP_004908
N-terminal Sequence Analysis	Ser27 & Ser29
Structure / Form	Pro form
Predicted Molecular Mass	26 kDa

SPECIFICATIONS

SDS-PAGE	33 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Boc-VPR-AMC (Catalog # ES011). The specific activity is >250 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	Lyophilized from 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 Tris, pH 9.0
- Recombinant Human Kallikrein 4/Prostase/EMSP1 (rhKLK4) (Catalog # 1719-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: BOC-Val-Pro-Arg-AMC (Catalog # ES011) , 90 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 rhKLK4 to 200 µg/mL in Activation Buffer.
2. Dilute Thermolysin to 2 µg/mL in Activation Buffer.
3. Combine equal volumes diluted rhKLK4 and Thermolysin and incubate at 37 °C for 2 hours to activate.
4. Stop the reaction with 1,10 Phenanthroline at a final concentration of 10 mM.
5. Dilute activated rhKLK4 to 2 ng/µL in Assay Buffer.
6. Dilute Substrate to 200 µM in Assay Buffer.
7. Load 50 µL of the 2 ng/µL rhKLK4 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 without any rhKLK4.
8. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
9. 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:

- rhKLK4: 0.100 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 µg/mL in sterile 50 mM Tris, 10 mM $CaCl_2$ and 150 mM NaCl, 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.

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BACKGROUND

Kallikrein 4 (KLK4), also known as prostase or enamel matrix serine protease 1 (EMSP1), is a serine protease of the human tissue kallikrein gene family (1). Among normal tissues, human KLK4 is specifically expressed in the prostate (2). It is over-expressed in prostate cancer and this expression is regulated by hormones including androgens, esterogen and progesterone (3). rhKLK4 readily activates pro-KLK3/PSA and pro-urokinase type plasminogen activator (uPA), indicating it may initiate events involving PSA and uPA in either normal or abnormal processes (4). KLK4 may have additional roles such as functioning as one of the two major enamel proteases identified that process enamel matrix proteins (5). In addition to being a secreted enzyme, it is also a nuclear protein (3, 6). The deduced amino acid sequence of human KLK4 consists of a signal peptide, a short pro region and a mature/active enzyme. rhKLK4 can be activated *in vitro* by thermolysin, a zinc protease. The peptidase activity can be inhibited by AEBSF (R&D Systems, Catalog # EI001), a general serine protease inhibitor.

References:

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