

## 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, estrogen 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:

1. Yousef, G.M. and E.P. Diamandis (2001) *Endocrine Rev.* **22**:184.
2. Nelson, P.S. *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96**:3114.
3. Xi, Z. *et al.* (2004) *Cancer Res.* **64**:2365.
4. Takayama, T.K. *et al.* (2001) *Biochemistry* **40**:15341.
5. Simmer, J.P. and J.C. Hu (2002) *Connect Tissue Res.* **43**:441.
6. Ryu, O.H. *et al.* (2002) *Eur. J. Oral. Sci.* **110**:358.

## Description

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

## Specifications

<b>SDS-PAGE</b>	33 kDa, reducing conditions
<b>Activity</b>	Measured by its ability to cleave the fluorogenic peptide substrate Boc-VPR-AMC R&D Systems, Catalog # ES011. The specific activity is > 250 pmoles/min/μg, as measured under the described conditions. See Activity Assay Protocol.
<b>Endotoxin Level</b>	<1.0 EU per 1 μg of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
<b>Formulation</b>	Lyophilized from a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

## Preparation and Storage

<b>Reconstitution</b>	Reconstitute at 200 μg/mL in sterile 50 mM Tris, 10 mM CaCl <sub>2</sub> and 150 mM NaCl, pH 7.5.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 6 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 3 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## Activity Assay Protocol

### Materials

- Activation Buffer: 50 mM Tris, 10 mM CaCl<sub>2</sub>, 150 mM NaCl, pH 7.5 (TCN)
- Assay Buffer: 50 mM Tris, pH 9.0
- Recombinant human Kallikrein 4 (rhKLK-4) (R&D Systems, Catalog # 1719-SE)
- Thermolysin (R&D Systems, Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: BOC-Val-Pro-Arg-AMC (R&D Systems, 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 rhKLK-4 to 200 μg/mL in Activation Buffer.
2. Dilute Thermolysin to 2 μg/mL in Activation Buffer.
3. Combine equal volumes diluted rhKLK-4 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 rhKLK-4 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 rhKLK-4 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 rhKLK-4.
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:

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmole/RFU)}}{\text{amount of enzyme } (\mu\text{g})}$$

\*Adjusted for Substrate Blank

\*\*Derived using calibration standard 7-Amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891)

### Final Assay Conditions Per Well

- rhKLK-4: 0.100 μg
- Substrate: 100 μM

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NOT FOR USE IN HUMANS