

Background

Kallikrein 3, commonly known as prostate specific antigen (PSA), is a serine protease of the human tissue Kallikrein gene family (1). PSA is synthesized in the ductal and acinar epithelium of the prostate gland and secreted into the seminal plasma in high concentrations (0.5 - 2 g/L) (2). A small portion of PSA "leaks" into the systemic circulation, the levels of which increase significantly (30-fold) from prostate cancer tissue than normal prostate tissue (3). PSA has become a well established tumor marker that aids the diagnosis, staging, and follow up of prostate cancer.

The deduced amino acid sequence of human PSA consists of a signal peptide, a short pro region and a mature/active enzyme. The pro-enzyme is activated, possibly by active Kallikreins 2, 4 or 15 *in vivo* (4). rhPSA is activated by thermolysin, a zinc protease. The active PSA cleaves several tyrosyl peptide bonds in semenogelins I and II, which are the major gel-forming proteins produced by the seminal vesicles (5). Several inhibitors including serpin A3/ α_1 -antichymotrypsin (ACT) and α_2 -macroglobulin are known to form complexes with PSA.

References:

1. Yousef, G.M. and E.P. Diamandis (2001) *Endocrine Rev.* **22**:184.
2. Ward, A.M. *et al.* (2001) *Ann. Clin. Biochem.* **38**:633.
3. Jain, S. *et al.* (2002) *Postgrad. Med. J.* **78**:646.
4. LiLja H. (2003) *Urology* **62**:27.
5. Takayama, T.K. *et al.* (1997) *J. Biol. Chem.* **272**:21582.

Description

Source	Murine myeloma cell line, NS0-derived Ala18 - Pro261, with a C-terminal 10-His tag Accession # P07288
N-terminal Sequence Analysis	Ala18
Structure / Form	Pro form
Predicted Molecular Mass	28 kDa

Specifications

SDS-PAGE	36 kDa, reducing conditions
Activity	Measured by its ability to cleave the colorimetric peptide substrate, Succinyl-Arg-Pro-Tyr-p-Nitroanilide (Suc-RPY-pNA). The specific activity is > 100 pmoles/min/ μ g, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μ g 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 μ m filtered solution in MES, NaCl and CaCl ₂ . See Certificate of Analysis for details.

Preparation and Storage

Reconstitution	Reconstitute at 200 μ g/mL in 50 mM Tris, 10 mM CaCl ₂ , 150 mM NaCl and 0.05% (w/v) Brij-35, 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. <ul style="list-style-type: none"> • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, 1 M NaCl, pH 8.0
- Recombinant human Kallikrein 3 (KLK3) (R&D Systems, Catalog # 1344-SE)
- Thermolysin (R&D Systems, Catalog # 3097-ZN)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Substrate: Suc-Arg-Pro-Tyr-pNa (AnaSpec, Catalog # 20586), 10 mM in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhKLK3 to 200 μ g/mL in Activation Buffer.
2. Dilute Thermolysin to 2 μ g/mL in Activation Buffer.
3. Combine equal volumes of 200 μ g/mL rhKLK3 and 2 μ g/mL Thermolysin.
4. Incubate at 37 °C for 5 minutes.
5. Dilute 1,10 Phenanthroline to 20 mM in Assay Buffer.
6. Stop Thermolysin activity by adding 1,10 Phenanthroline to a final concentration of 10 mM.
7. Dilute activated rhKLK3 to 20 ng/ μ L in Assay Buffer.
8. Dilute Substrate to 2 mM in Assay Buffer.
9. Load 50 μ L of the 20 ng/ μ L rhKLK3 in a plate and start the reaction by adding 50 μ L of 2 mM Substrate. Include a Substrate Blank containing 50 μ L Assay Buffer and 50 μ L of 2 mM Substrate.
10. Read at a wavelength of 405 nm (bottom read) in kinetic mode for 5 minutes.
11. Calculate specific activity:

$$\text{Specific Activity (pmoles/min}/\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 8800 M⁻¹cm⁻¹

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions Per Well

- rhKLK3: 1 μ g
- Substrate: 1 mM

2/9/2010

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NOT FOR USE IN HUMANS