Recombinant Human Kallikrein 3/PSA  
Catalog Number: 1344-SE

DESCRIPTION

Source
Mouse 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 >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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation
Lyophilized from a 0.2 µm filtered solution in Tris, NaCl and CaCl₂. See Certificate of Analysis for details.

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/PSA (rhKLK3) (Catalog # 1344-SE)  
- Bacterial Thermolysin (Thermolysin) (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 µg/mL in Activation Buffer.  
2. Dilute Thermolysin to 2 µg/mL in Activation Buffer.  
3. Combine equal volumes of 200 µg/mL rhKLK3 and 2 µg/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/µL in Assay Buffer.  
8. Dilute Substrate to 2 mM in Assay Buffer.  
9. Load 50 µL of the 20 ng/µL rhKLK3 in a plate and start the reaction by adding 50 µL of 2 mM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 2 mM Substrate.  
10. Read at a wavelength of 405 nm (bottom read) in kinetic mode for 5 minutes.
11. Calculate specific activity:

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

*Adjusted for Substrate Blank  
**Using the extinction coefficient 8800 M⁻¹ cm⁻¹  
***Using the path correction 0.32 cm  
Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions
Per Well:
- rhKLK3: 1 µg  
- Substrate: 1 mM

PREPARATION AND STORAGE

Reconstitution
Reconstitute at 200 µg/mL in 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl and 0.05% (w/v) Brij-35, pH 7.5.

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 reconstitution.

DATA

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Enzyme Activity

Recombinant Human Kallikrein 3 (Catalog # 1344-SE) is measured by its ability to cleave the colorimetric peptide substrate, Succinyl-Arg-Pro-Tyr-p-Nitroanilide (Suc-RPY-pNA).

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/α1-antichymotrypsin (ACT) and α2-macroglobulin are known to form complexes with PSA.

References: