

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived
Ile67-Ser293, with a C-terminal 10-His tag
Accession # Q9Y337

N-terminal Sequence Analysis Ile67

Structure / Form Mature form

Predicted Molecular Mass 31 kDa

SPECIFICATIONS

SDS-PAGE 40 kDa, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate Boc-VPR-AMC R&D Systems, Catalog # ES011. The specific activity is >200 pmol/min/μg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com

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 Supplied as a 0.2 μm filtered solution in MES, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M NaH₂PO₄, pH 8.0
- Recombinant Human Kallikrein 5 (rhKLK5) (Catalog # 1108-SE)
- Fluorogenic Peptide Substrate BOC-Val-Pro-Arg-AMC (Catalog # ES011), 10 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 rhKLK5 to 2 ng/μL in Assay Buffer.
2. Dilute Substrate to 200 μM in Assay Buffer.
3. Load 50 μL of the 2 ng/μL rhKLK5 into 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 rhKLK5.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

Final Assay Conditions Per Well

- rhKLK5: 0.1 μg
- Substrate: 100 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.

BACKGROUND

Human tissue Kallikrein 5 (hK5, also known as stratum corneum tryptic enzyme) is a serine protease of the human tissue Kallikrein gene family (2). The deduced amino acid sequence of human KLK5 consists of a signal peptide (Met1 to Gly22), a pro region (Val23 to Arg66) and a mature/active enzyme (Ile67 to Ser293). KLK5 is expressed mainly in skin, breast, brain and testis and to a lesser extent in many other tissues (3). KLK5 overexpression is an indicator of poor prognosis in ovarian cancer (4). hK5 is a candidate physiological activator of hK7 in the stratum corneum (5). Indeed, rhK5 can be used to activate rhK7 (R&D Systems, Catalog # 2624-SE) by incubating rhK5 with recombinant human (rh) K7 (both at 0.1 mg/mL) in 25 mM MES, 0.15 M NaCl, pH 5.5, at 37 °C overnight.

rhK5 enzymatic activity can be inhibited by rhSerpinA1, A5, F2, and G1 (R&D Systems, Catalog # 1268-PI, 1266-PI, 1470-PI, 2488-PI).

References:

1. Brattsand, M. and T. Egelrud (1999) J. Biol. Chem. **274**:30033.
2. Yousef, G.M. *et al.* (1999) Anticancer Res. **19**:2843.
3. Yousef, G.M. and E.P. Diamandis (2001) Endocrine Rev. **22**:184.
4. Yousef, G.M. and E.P. Diamandis (1999) J. Biol. Chem. **274**:37511.
5. Kim, H. *et al.* (2001) Br. J. Cancer **84**:643.