Recombinant Human Kallikrein 8/Neuropsin

Catalog Number: 2025-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Gln29-Gly260, with a C-terminal 10-His tag Accession # O60259
N-terminal Sequence Analysis	No results obtained: Gln29 predicted
Structure / Form	Pro form
Predicted Molecular Mass	26 kDa

SPECIFICATIONS

SDS-PAGE	37-39 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Boc-VPR-AMC (Catalog # ES011). The specific activity is >500 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	Supplied as 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, pH 8.0
- Assay Buffer: 50 mM Tris, pH 9.0
- Recombinant Human Kallikrein 8/Neuropsin (rhKLK8) (Catalog # 2025-SE)
- Lysyl-Endopeptidase (Wako BioProducts, Catalog # 129-02541), 20 U/mL in PBS
- 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 rhKLK8 to 200 µg/mL in Activation Buffer.
2. Dilute Lysyl-Endopeptidase to 0.4 mU/mL in Activation Buffer.
3. Activate rhKLK8 by combining equal volumes of diluted rhKLK8 and Lysyl-Endopeptidase.
4. Incubate at 37 °C for 30 minutes.
5. Dilute activated rhKLK8 to 1 ng/µL in Assay Buffer.
6. Dilute Substrate to 200 µM in Assay Buffer.
7. Load 50 µL of 1 ng/µL rhKLK8 into the plate, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
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 (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhKLK8: 0.050 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.

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BACKGROUND

Kallikrein 8 (KLK8), also known as neuropsin or ovasin, is a member of the human tissue kallikrein family (1). Two alternatively spliced forms exist, resulting in 260 (isoform 1) and 305 (isoform 2) amino acid sequences, respectively (2). Isoform 1 consists of a signal peptide (residues 1 to 28), a short pro peptide (residues 29 to 32) and the mature chain (residues 33 to 260). Isoform 2 is identical to isoform 1, except that a 45 amino acid segment is inserted in isoform 2 between residues 23 and 24 in isoform 1. Isoform 1 is predominantly expressed in pancreas whereas isoform 2 is preferentially expressed in adult brain and hippocampus, although both forms are expressed in fetal brain and placenta in comparable levels. The brain function of KLK8 seems evident in neuropsin knockout mice that showed abnormalities of synapses and neurons and predisposation to global seizure activity (3, 4). KLK8 is a novel marker for ovarian and cervical cancer carcinomas (5, 6). Recombinant human KLK8, after being activated by lysyl endopeptidase, can cleave fibronectin and several small peptide substrates (7, 8). This activity can be inhibited by recombinant human Serpin A5, recombinant human Serpin F2 and AEBSF (Catalog # 1266-PI, 1470-PI and EI001, respectively). Recombinant human KLK8 produced by R&D Systems corresponds to isoform 1.

References:

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