Recombinant Human Kallikrein 2 His-tag

Catalog Number: 11691-SE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Kallikrein 2 protein Ile25-Pro261, with a C-terminal 10-His tag Accession # P20151.1
N-terminal Sequence Analysis	Ile25
Predicted Molecular Mass	28 kDa

SPECIFICATIONS

SDS-PAGE	22-32 kDa, under non-reducing conditions
Activity	Measured by its ability to cleave a flourogenic peptide substrate Pro-Phe-Arg-7-amido-4-methylcoumarin (PFR-AMC). The specific activity is >250 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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 Sodium Citrate and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Recombinant Human Kallikrein 2 (rhKLK2) (Catalog # 11691-SE)
- Substrate: Pro-Phe-Arg-AMC, 10 mM stock in DMSO
- Black 96-well plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhKLK2 to 4 µg/mL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. In a plate, load 50 µL of 4 µg/mL rhKLK2, and start the reaction by adding 50 µL of the 200 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
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:

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

*Adjusted for Substrate Blank
**Derived using calibration standard 7-Amino, 4-Methyl Coumarin.

Final Assay Conditions

Per Well:

- rhKLK2: 0.2 µg
- Substrate: 100 µM

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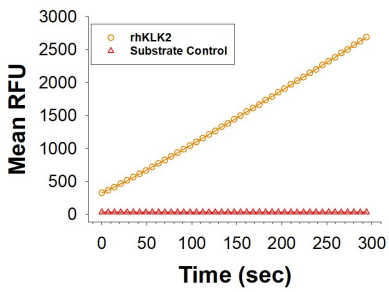
PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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.

DATA

Enzyme Activity



Recombinant Human Kallikrein 2 His-tag Protein Enzyme Activity. Recombinant Human Kallikrein 2 His-tag (Catalog # 11691-SE) is measured by its ability to cleave the fluorogenic peptide substrate Pro-Phe-Arg-7-amido-4-methylcoumarin (PFR-AMC).

BACKGROUND

Recombinant human Kallikrein 2 (KLK2) is also known as glandular kallikrein or the prostate-specific glandular kallikrein as it is structurally related to KLK3, the prostate-specific antigen (PSA). KLK2 is a secreted serine protease member of the tissue kallikrein subfamily family of the peptidase S1 family (1). It is highly expressed in the human prostate gland (2) and synthesized as a pre-proenzyme like other kallikriens. Removal of the propeptide occurs through cleavage by trypsin-like activity to activate the protein (1). KLK2 also contains a catalytic domain with an extended 99 or kallikrein loop (3) and glycan that regulate its activity (4). KLK2 is highly specific for cleavage after arginine residues and is able to autoactivate and play a role in activation of KLK cascades (5). In addition, KLK2 has been reported to activate other key proteins including the urokinase-type plasminogen activator (6), IGFBPs (7), and IL10 (8). Its activity is highly regulated by activation cascades, endogenous inhibitors such as serpins protein C inhibitor, antichymotrypsin, and plasminogen activator inhibitor 1 (9-11), pH, and metal dependency (1). It is heavily implicated to play a role in prostate cancer and is attractive as a biomarker and therapeutic target (12-15).

References:

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