Recombinant Mouse Kininogen High Molecular Weight (HKa)

Catalog Number: 2206-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Glu21-Ser661, with a C-terminal 10-His tag Accession # O08677
N-terminal Sequence Analysis	Glu21 (mature and heavy chains) & Ser389 (light chain)
Predicted Molecular Mass	72 kDa (mature), 38 kDa (heavy) & 31 kDa (light)

SPECIFICATIONS

SDS-PAGE	120 kDa, 60 kDa, 48 kDa, reducing conditions
Activity	Measured by its ability to inhibit papain cleavage of a fluorogenic peptide substrate Z-FR-AMC (Catalog # ES009). The IC_{50} value is <5 nM, 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 under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from 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, 5 mM DTT, pH 7.0
- Assay Buffer: 50 mM Tris, pH 7.0
- Recombinant Mouse Kininogen High Molecular Weight (HKa) (rmKininogen) (Catalog # 2206-PI)
- Papain (Sigma, Catalog # P4762)
- Substrate: Z-Phe-Arg-AMC (Catalog # ES009), 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 Papain to 100 µg/mL in Activation Buffer.
2. Incubate at room temperature for 10 minutes.
3. Prepare a dilution curve of rmKininogen (MW: 72,313 Da) in Assay Buffer. Make the following serial dilutions: 1000, 500, 125, 62.5, 41.7, 27.8, 18.5, 9.26, 3.09, and 1.03 nM.
4. Dilute activated Papain to 2.4 µg/mL in Assay Buffer.
5. Mix equal volumes of the rmKininogen curve dilutions and the diluted active Papain. Include a control (in duplicate) containing Assay Buffer and the diluted active Papain.
6. Incubate mixtures at room temperature for 10 minutes.
7. Perform a 1/5 dilution with Assay Buffer to the incubated mixtures.
8. Dilute Substrate to 200 µM in Assay Buffer.
9. Load 50 µL of diluted incubated mixture into a plate, and start the reaction by adding 50 µL of 200 µM Substrate.
10. Read at excitation and emission wavelengths of 380 nm and 460 nm, respectively, for 5 minutes in kinetic mode.
11. Derive the 50% inhibiting concentration (IC_{50}) value for rmKininogen by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
12. The specific activity for papain at each point may be determined using the following formula (if needed):

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- Papain: 0.012 µg
- Substrate: 100 µM
- rmKininogen curve: 50, 25, 6.25, 3.125, 2.09, 1.39, 0.925, 0.463, 0.155, and 0.052 nM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM Tris and 150 mM NaCl, 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.

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BACKGROUND

Kininogen, also known as α2-thiol proteinase inhibitor, is a multi-function protein. There are two alternatively spliced forms, designated as the high molecular weight (HMW) and low MW (LMW) forms (1). The mouse HMW form is synthesized as a 661 amino acid residue precursor with a signal peptide (residues 1-20). The mature chain (residues 21-661) is further processed into the heavy (residues 21-379) and the light (residues 389-661) chains. The active peptide bradykinin (residues 380-388) is released, which has a variety of functions including muscle contraction, hypotension and inflammation. The heavy chain consists of three cystatin-like domains, which are responsible for inhibiting cysteine proteases. The light chain consists of a His-rich domain, which is associated with the clotting activity. In comparison to the HMW form, the LMW Kininogen (432 residues) has the same sequence in its heavy chain and bradykinin, but a different sequence in its light chain (residues 401-432). The LMW form is not involved in blood clotting.

References:

1. Takano, M. *et al*. (1997) Biochim. Biophys. Acta **1352**:222.

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