R&D SYSTEMS
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# Recombinant Human Kininogen High Molecular Weight (HKa)

Catalog Number: 1569-PI

## DESCRIPTION

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|---|---|
| **Source** | Mouse myeloma cell line, NS0-derived<br>Gln19-Ser644, with an N-terminal signal sequence and a C-terminal 10-His tag<br>Accession # P01042 |
| **N-terminal Sequence Analysis** | Ser390 (light chain) & Lys438 (minor species) with Gln19 predicted to be present and blocked |
| **Structure / Form** | High molecular weight form consisting of the mature chain, the heavy and light chains, and a minor species |
| **Predicted Molecular Mass** | 71 kDa (mature), 41 kDa (heavy) & 30 kDa (light) |

## SPECIFICATIONS

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| **SDS-PAGE** | 120 kDa, 60 kDa and 48 kDa, reducing conditions |
| **Activity** | Measured by its ability to inhibit papain cleavage of a fluorogenic peptide substrate Z-FR-AMC (Catalog # ES009).<br>The $IC_{50}$ value is <3 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 Sodium Acetate 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 Human Kininogen High Molecular Weight (HKa) (rhKininogen) (Catalog # 1569-PI)
- Papain (Sigma, Catalog # P4762)
- Substrate: Z-Phe-Arg-AMC (R&D Systems, 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. Chill Activation Buffer on ice.
2. Dilute Papain to 100 μg/mL in Activation Buffer.
3. Incubate at room temperature for 10 minutes.
4. Prepare a dilution curve of rhKininogen (MW: 71,211Da) in Assay Buffer. Make the following serial dilutions: 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 nM.
5. Dilute activated Papain to 2 μg/mL in Assay Buffer.
6. Mix equal volumes of the rhKininogen curve dilutions and the diluted active Papain. Include a control (in duplicate) containing Assay Buffer and the diluted active Papain.
7. Incubate mixtures at room temperature for 10 minutes.
8. Dilute Substrate to 200 μM in Assay Buffer.
9. Perform a 1/5 dilution with Assay Buffer to the incubated mixture of rhKininogen and Papain.
10. Load 50 μL of diluted incubated mixture into the plate, and start the reaction by adding 50 μL of 200 μM Substrate.
11. Read at excitation and emission wavelengths of 380 nm and 460 nm, respectively, for 5 minutes in kinetic mode.
12. Derive the 50% inhibition concentration ($IC_{50}$) for rhKininogen by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
13. 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: 0.010 μg
- Substrate: 100 μM
- rhKininogen curve: 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 nM

## PREPARATION AND STORAGE

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| **Reconstitution** | Reconstitute at 100 μg/mL in sterile 25 mM Tris and 100 mM NaCl, pH 7.5. |
| **Shipping** | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>• 6 months from date of receipt, -20 to -70 °C as supplied.<br>• 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 HMW form is synthesized as a 644 amino acid residue precursor with a signal peptide (residues 1-18). The mature chain (residues 19-644) is further processed into the heavy (residues 19-380) and the light (residues 390-644) chains. The active peptide bradykinin (residues 381-389) 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 (427 residues) has the same sequence in its heavy chain and bradykinin, but a different sequence in its light chain (residues 402-427). The LMW form is not involved in blood clotting.

**References:**

1. Takagaki, Y. *et al*. (1985) J. Biol. Chem. **260**:8601.



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