R&D SYSTEMS
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# Human $\alpha_2$-Macroglobulin

Catalog Number: 1938-PI

## DESCRIPTION

| | |
|---|---|
| **Source** | Human plasma-derived<br>The human plasma used for the isolation of this product was certified by the supplier to be HIV-1 and HBsAg negative at the time of shipment. Human blood products should always be treated in accordance with universal handling precautions. |
| **N-terminal Sequence Analysis** | Ser24 |
| **Structure / Form** | Disulfide-linked homo-oligomer |

## SPECIFICATIONS

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|---|---|
| **SDS-PAGE** | 90-170 kDa, reducing conditions |
| **Activity** | Measured by its ability to trap trypsin. The trapped trypsin is no longer able to interact with protein substrates or inhibitors, but still able to cleave small peptide substrates or inhibitors.<br>Human α2-Macroglobulin has an $IC_{50}$ value of <5 nM, as measured under the described conditions. |
| **Endotoxin Level** | <1.0 EU per 1 µg of the protein by the LAL method. |
| **Purity** | >90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining. |
| **Formulation** | Supplied as a 0.2 µm filtered solution in $NaH_2PO_4$, NaCl and Glycerol. See Certificate of Analysis for details. |

## Activity Assay Protocol

**Materials**

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Human $\alpha_2$-Macroglobulin (h$\alpha_2$-Macroglobulin) (Catalog # 1938-PI)
- Recombinant Human Serpin F2 (rhSerpin F2) (Catalog # 1470-PI)
- Recombinant Human Active Trypsin 3/PRSS3 (rhTrypsin 3) (Catalog # 3714-SE)
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-$NH_2$ (Catalog # ES002)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

**Assay**

1. Dilute rhTrypsin 3 to 1.28 µg/mL with Assay Buffer.
2. Prepare a curve of h$\alpha_2$-Macroglobulin with Assay Buffer. Dilute h$\alpha_2$-Macroglobulin (MW: 720000 Da) to the following concentrations: 200 nM, 100 nM, 50 nM, 25 nM, 12.5 nM, 6.25 nM, 3.125 nM, and 0.3125 nM.
3. Combine 30 µL of h$\alpha_2$-Macroglobulin curve with 30 µL of 1.28 µg/mL rhTrypsin 3. Include a control containing 30 µL of Assay Buffer with 30 µL of rhTrypsin 3 in duplicate. Also include a h$\alpha_2$-Macroglobulin control in duplicate for each sample tested containing 30 µL of 200 nM h$\alpha_2$-Macroglobulin and 30 µL Assay Buffer.
4. Incubate at 37 °C for 1 hour.
5. Dilute rhSerpin F2 to 40 µg/mL with Assay Buffer.
6. Add 60 µL of 40 µg/mL rhSerpin F2 to each reaction.
7. Incubate at 37 °C for 15 minutes.
8. Dilute Substrate to 20 µM in Assay Buffer.
9. Load into plate 50 µL of h$\alpha_2$-Macroglobulin curve containing rhTrypsin 3 and rhSerpin F2, and start the reaction by adding 50 µL of 20 µM Substrate to each well.
10. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration ($IC_{50}$) of h$\alpha_2$-Macroglobulin, by its trapping of rhTrypsin 3, toward rhSerpin F2 activity by plotting RFU/min (or specific activity) vs. concentration (h$\alpha_2$-Macroglobulin) with 4-PL fitting.
12. The specific activity for rhTrypsin 3 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 h$\alpha_2$-Macroglobulin control

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

**Final Assay Conditions**

Per Well:

- h$\alpha_2$-Macroglobulin: 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, and 0.0391 nM
- rhTrypsin 3: 0.016 µg
- rhSerpin F2: 1 µg
- Substrate: 10 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

Human $\alpha_2$-macroglobulin (hα2M) is a serum glycoprotein that has sequence similarity to other members of the α2M family including complement components C3, C4 and C5 (1). α2M is synthesized as a polypeptide of 1474 amino acids with a signal peptide (23 residues) (2). The mature protein is a tetramer (720 kDa) of 4 identical subunits (180 kDa), which form two disulfide bond-linked dimers. As a general and irreversible protease inhibitor implicated in many processes, α2M is able to inhibit all four classes of proteases by a unique trapping mechanism. The bait region of hα2M (residues 690-728) contains specific cleavage sites for different proteases. The cleavage of the bait region by a protease induces a conformation change in α2M, which then traps and forms a covalent bond with the protease. The trapped protease remains active against small peptide substrates but loses its ability to interact with large protein substrates or inhibitors.

**References:**

1. Sottrup-Jensen, L. *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:9.
2. Kan, C.C. *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:2282.



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