Rat Alpha-2-Macroglobulin ELISA Kit

Enzyme Immunoassay for the quantification of alpha-2-Macroglobulin in plasma, serum, urine, and cell culture samples

Catalog number: ARG81189

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INTRODUCTION

Alpha-2-macroglobulin is a protease inhibitor and cytokine transporter. It inhibits many proteases, including trypsin, thrombin and collagenase. A2M is implicated in Alzheimer disease (AD) due to its ability to mediate the clearance and degradation of A-beta, the major component of beta-amyloid deposits. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of rat alpha-2-Macroglobulin in plasma, serum, urine, and cell culture samples.

This assay employs the sandwich enzyme immunoassay technique. A antibody specific for rat alpha-2-Macroglobulin has been pre-coated onto a microtiter plate. Rat alpha-2-Macroglobulin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for alpha-2-Macroglobulin. The biotinylated antibody interacts with streptavidin-horseradish peroxidase which catalyzes the substrate solution. The reaction is monitored by a color change which is readable at OD of 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards. The intensity of color development is proportional to the amount of alpha-2-Macroglobulin in the samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Microtiter Plate	12 x 8 wells	4°C

20X wash buffer concentrate	30ml X 2 bottles	4°C
Plate sealer	3 pieces	4°C
Biotinylated rat alpha-2-Macroglobulin antibody (50X)	1 vial (140 µl)	-20°C
Rat alpha-2-Macroglobulin Standard	1 vial (160ng, lyophilized)	4°C (store at -20°C after reconstitution)
EIA Diluent (10X)	30 ml	4°C
Streptavidin-HRP (100X)	80 µl	-20°C
Substrate Solution (TMB)	8 ml	4°C, ready for use
STOP solution	12 ml	4°C, ready for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the components at recommended temperature.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines.

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect supernatants. Dilute samples 1:1000 into EIA Diluent and assay. The undiluted samples can be stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Dilute samples 1:1000 into EIA Diluent and assay. The undiluted samples can be stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants: Collect cell culture media and centrifuge at 3000 x g for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:2 into EIA Diluent and assay. The undiluted samples can be stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- EIA Diluent: Dilute 10X EIA Diluent into distilled water to yield 1X Wash

buffer. If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or until crystals disappear. Mix well before use. Store for up to 30 days at 2-8°C.

- **Biotinylated rat alpha-2-Macroglobulin antibody**: Briefly spin down tube. Reconstitute the antibody 1:50 with 1X EIA Diluent buffer. Any remaining solution should be frozen at-20°C.
- **Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or until crystals disappear. Mix well before use.
- **Streptavidin-HRP conjugate**: Spin down the Streptavidin-HRP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at-20°C.
- **Standard curve**: Reconstitute the Standard vial with 4 ml of 1X EIA buffer. Vortex. The concentration of this stock solution is 40 ng/ml. Allow to sit for 10 minutes at RT. Mix well and spin down before use. Dilute standard solutions according to the table below and make serial dilutions. EIA Diluent serves as the zero standard (0 ng/ml).

Tube No.	Standard	1X EIA Buffer	Concentrations (ng/ml)
1	1 part of standard stock	3 parts	10
2	1 part of Tube 1	1 part	5
3	1 part of Tube 2	1 part	2.5
4	1 part of Tube 3	1 part	1.25
5	1 part of Tube 4	1 part	0.625
6	1 part of Tube 5	1 part	0.313
7	1 part of Tube 6	1 part	0.156

ASSAY PROCEDURE

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
2. Add 50µl of rat alpha-2-Macroglobulin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
3. Remove sealer from plate.
4. Aspirate each well and wash, repeating the process 1 times for a total 2 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 50 µl Biotinylated rat alpha-2-Macroglobulin antibody into each well.
6. Reseal the plate with sealer. Incubate for 1 hour at RT.
7. Wash as according to step 4.
8. Add 50 µl Streptavidin-HRP solution into each well.
9. Reseal the plate with sealer. Incubate for 30 minutes at RT.
10. Wash as according to step 4.
11. Add 50 µl TMB substrate solution into each well.

12. Incubate for 15 minutes at RT. (Protect from light)
13. Add 50 µl STOP solution into all wells to stop the reaction.
14. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

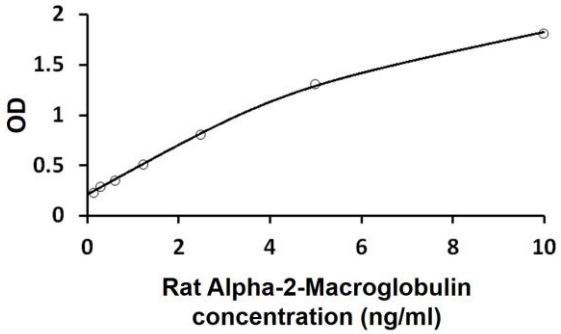
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Range

Standard Range: 0.156- 10 ng/ml

Recovery

83-111%

Linearity

Sample Dilution	Plasma	Serum
1:100000	89%	88%
1:200000	99%	98%
1:400000	107%	108%

Cross Reactivity

The kit cross-reacts:

alpha-2-Macroglobulin (rat) 100%

This kit detects no cross-reactivity with the followings:

alpha-2-Macroglobulin (Human)

alpha-2-Macroglobulin (Mouse)

alpha-2-Macroglobulin (Rabbit)

alpha-2-Macroglobulin (Canine)

alpha-2-Macroglobulin (Swine)

alpha-2-Macroglobulin (Monkey)