ELISA PRODUCT INFORMATION & MANUAL

Alpha-Macroglobulin

NBP2-60637

Enzyme-linked Immunosorbent Assay for quantitative detection of Rat alpha-Macroglobulin. For research use only.

Not for diagnostic or therapeutic procedures.

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Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 15 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Rat alpha-Macroglobulin ELISA Kit

Catalog No. NBP2-60637

Sample insert for reference use only

Introduction

Alpha-macroglobulin is a major serum protein with diverse functions, including inhibition of protease activity and binding of growth factors, cytokines, and disease factors (1).

Principle of the Assay

The Rat alpha-Macroglobulin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat alpha-macroglobulin in rat **plasma, serum, urine, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures rat alpha-macroglobulin in less than 4 hours. A polyclonal antibody specific for rat alpha-macroglobulin has been pre-coated onto a 96-well microplate with removable strips. Rat alpha-macroglobulin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for rat alpha-macroglobulin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Rat alpha-Macroglobulin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat alpha-macroglobulin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Rat alpha-Macroglobulin Standard:** Rat alpha-macroglobulin in a buffered protein base (160 ng, lyophilized).
- **Biotinylated Rat alpha-Macroglobulin Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against rat alpha-macroglobulin (140 µl).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **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 use supernatants. Dilute samples 1:200000 with EIA Diluent or within the range of 1:50000 to 1:500000, and assay. Depending on application needs, user should determine proper dilutions. 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 1:200000 with EIA Diluent or within the range of 1:50000 to 1:500000, and assay. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes and assay. Dilute samples 1:100 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

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 160 ng of Rat alpha-Macroglobulin Standard with 4 ml of EIA Diluent to generate a 40 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The standard stock solution (40 ng/ml) should be further diluted 1:4 with EIA Diluent to produce a 10 ng/ml standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (10 ng/ml) 1:2 with equal volume of EIA Diluent to produce 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within the next 30 days.

Standard Point	Dilution	[Rat AMG] (ng/ml)
P1	1 part Standard (40 ng/ml) + 3 parts EIA Diluent	10.00
P2	1 part P1 + 1 part EIA Diluent	5.000
P3	1 part P2 + 1 part EIA Diluent	2.500
P4	1 part P3 + 1 part EIA Diluent	1.250
P5	1 part P4 + 1 part EIA Diluent	0.625
P6	1 part P5 + 1 part EIA Diluent	0.313
P7	1 part P6 + 1 part EIA Diluent	0.156
P8	EIA Diluent	0.000

- **Biotinylated Rat alpha-Macroglobulin Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- 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.
- Add 50 µl of Rat alpha-Macroglobulin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Rat alpha-Macroglobulin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.

- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for 15 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

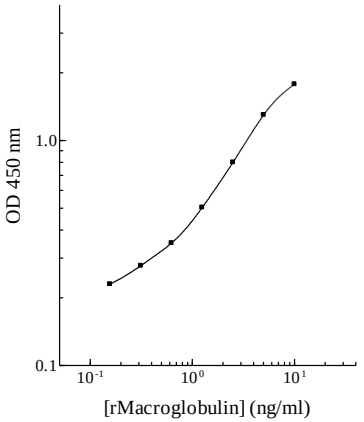
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

R. Macroglobulin Standard Curve



Performance Characteristics

- The minimum detectable level of rat alpha-macroglobulin is typically ~ 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.2% respectively.

Linearity

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1:100000	89%	88%
1:200000	99%	98%
1:400000	107%	108%

Recovery

Standard Added Value	0.25 – 2.5 ng/ml
Recovery %	83 – 111%
Average Recovery %	96%

Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Monkey	None
Human	None
Rat	100%
Swine	None
Rabbit	None
Mouse	None

Version 2.0R1