Mouse alpha-Macroglobulin ELISA Kit (Cell Culture Supernatants)

Introduction

Alpha-2-Macroglobulin is a major serum protein with diverse functions, including inhibition of protease activity and binding of growth factors, cytokines, and disease factors (1). Increased serum alpha-2-Macroglobulin has been suggested to be associated with multiple sclerosis (MS) (2), glomerular disease (3), and with liver diseases (4).

Mouse alpha-Macroglobulin (M-AMG) is believed to be a functional homologue of human alpha 2-Macroglobulin (h-alpha 2M).

Principal of the Assay

The Mouse alpha Macroglobulin ELISA kit is designed for detection of mouse alpha Macroglobulin in cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures alpha Macroglobulin in 4 hours. A polyclonal antibody specific for alpha Macroglobulin has been pre-coated onto a microplate. Alpha Macroglobulin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for alpha Macroglobulin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then 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 kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Alpha Macroglobulin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against alpha Macroglobulin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Alpha Macroglobulin Standard:** Mouse alpha Macroglobulin in a buffered protein base (20 ng, lyophilized).
- **Biotinylated alpha Macroglobulin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against mouse alpha Macroglobulin (80 µl).

- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 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

- Store kit at 2-8^{0}C or - 20^{0}C upon arrival up to the expiration date
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

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

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 20 ng of mouse alpha Macroglobulin Standard with 1 ml of MIX Diluent to generate a stock solution of 20 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the alpha Macroglobulin standard solution (20 ng/ml) 1:4 with MIX Diluent to produce 5, 1.25, 0.313 and 0.078 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Alpha Macroglobulin] (ng/ml)
P1	1 part Standard (20 ng/ml)	20.000
P2	1 part P1 + 3 part MIX Diluent	5.000
P3	1 part P2 + 3 part MIX Diluent	1.250
P4	1 part P3 + 3 part MIX Diluent	0.313
P5	1 part P4 + 3 part MIX Diluent	0.078
P6	MIX Diluent	0.000

- **Biotinylated Alpha Macroglobulin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** 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 MIX Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

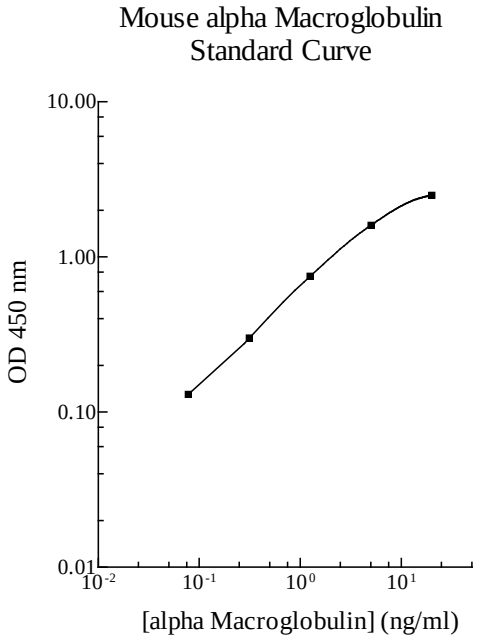
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated Alpha Macroglobulin Antibody to each well and incubate for one hour.
- Wash five times with 200 µl of Wash Buffer as 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 five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 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**. 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 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.



Performance Characteristics

- The minimum detectable level of Alpha Macroglobulin is typically < 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1 % and 7.0% respectively.

Recovery

Standard Added Value	0.5 – 5 ng/ml
Recovery %	80-110 %
Average Recovery %	95 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Monkey	None
Human	None
Rat	< 10
Swine	< 1
Rabbit	None

References

(1) Pineda-Salgado L *et al* (2005) Gene *Expr Patterns*. 6(1): 3-10
(2) Jensen PE *et al* (2004) *Biochim Biophys Acta*. 5; 1690(3): 203-7
(3) Yang AH *et al* (1997) *Nephrol Dial Transplant*. 12(3): 465-9
(4) Shiota G *et al* (1995) *J Med*. 26(5-6): 295-308

Version 1.1