AssayMax Human alpha2-Macroglobulin ELISA Kit

Catalog # EM1115-1

Introduction

Alpha2-Macroglobulin (alpha2M) 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 alpha2M has been suggested to be associate with multiple sclerosis (MS) (2); glomerular disease (3), and with liver diseases (4).

Principal of the Assay

The AssayMax Human alpha2-Macroglobulin ELISA kit is designed for detection of human alpha2-Macroglobulin in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique which measures alpha2-Macroglobulinin 4 hours. A polyclonal antibody specific for alpha2-Macroglobulin has been pre-coated onto a microplate. Alpha2-Macroglobulin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for alpha2-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

- **Alpha2-Macroglobulin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against alpha2-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.
- **Alpha2-Macroglobulin Standard:** Human alpha2-Macroglobulin in a buffered protein base (6 µg, lyophilized).
- **Biotinylated alpha2-Macroglobulin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human alpha2-Macroglobulin (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl)

- **MIX Diluent Concentrate (10x):** A 10-fold buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard 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, 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 2000 x g for 10 minutes and assay. Dilute samples 1:16000 with MIX Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:16000 into MIX Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **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.
- **Standard Curve:** Reconstitute the 6 µg of Human alpha2-Macroglobulin Standard with 3 ml of MIX Diluent to generate a stock solution of 2 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the alpha2-Macroglobulin standard solution (2 µg/ml) 1:4 with MIX Diluent to produce 0.5, 0.125, 0.031, 0.008 and 0.002 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml).

Standard Point	Dilution	[Alpha2-Macroglobulin] (μg/ml)
P1	1 part Standard (2 μg/ml)	2.000
P2	1 part P1 + 3 part MIX Diluent	0.500
P3	1 part P2 + 3 part MIX Diluent	0.125
P4	1 part P3 + 3 part MIX Diluent	0.031
P5	1 part P4 + 3 part MIX Diluent	0.008
P6	1 part P5 + 3 part MIX Diluent	0.002
P7	MIX Diluent	0.000

- **MIX Diluent Concentrate (10x):** Dilute MIX Diluent Concentrate 1:10 with reagent grade water.
- **Biotinylated alpha2-Macroglobulin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent.
- **Wash Buffer Concentrate (10x):** Dilute Wash Buffer Concentrate 1:10 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.

Assay Procedure

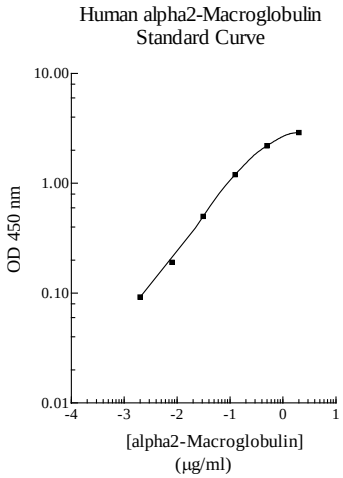
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. 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 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 complete remove liquid at each step.
- Add 50 μl of Biotinylated Alpha2-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 8 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 4-parameter or log-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma or serum mean value by the dilution factor of 16000.

Standard Curve

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



Precision, Sensitivity and Specificity

- The minimum detectable level of alpha2-Macroglobulin is typically < 2 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1 % and 7.0% respectively.
- No significant cross-reactivity or interference was observed.

Recovery

Standard Added Value	0.01 – 0.5 ug
Recovery %	80-115 %
Average Recovery %	97.5 %

Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
1:4000	100%	97%
1:8000	100%	101%
1:16000	105%	102%

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

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