



Alpha 2-Macroglobulin (Rat) ELISA

For quantitative determination of Alpha 2-Macroglobulin in serum and plasma of rats.

**Please see Appendix A for Reference Serum information
For Research Use Only. Not For Use In Diagnostic Procedures.**

Catalog Number: 41-A2MRT-E01

Size: 96 Wells

Version: 4.1 -ALPCO – 4.0

INTENDED USE

The Alpha 2-Macroglobulin ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the determination of Alpha 2-Macroglobulin in rat serum and plasma. For research use only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the alpha 2-macroglobulin (A2M) present in samples reacts with the anti-A2M antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-A2M antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound A2M. Following another washing step, the bound enzyme is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of A2M in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of alpha 2-macroglobulin in the test sample. The quantity of A2M in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

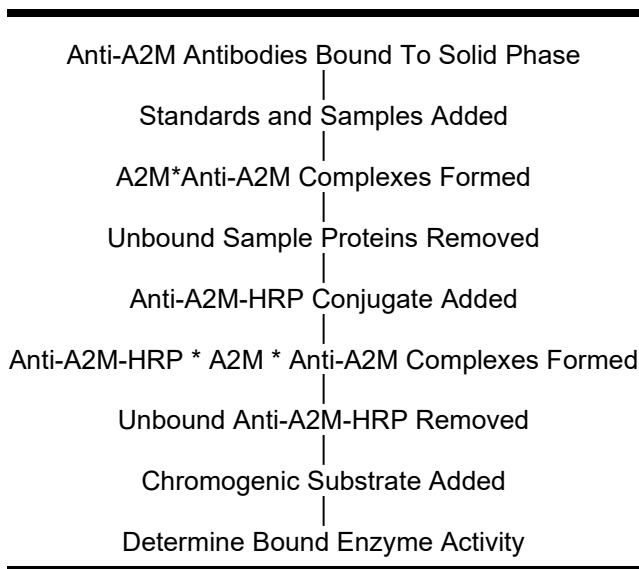


Figure 1.

LIMITATION OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

KIT COMPONENTS

The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date when stored and used per this kit protocol insert.

Component	Description	Preparation	Storage	Stability
ELISA Microplate, antibody coated	One plate of 12 removable 8 well strips, antibody coated	Ready to use as supplied.	2-8°C, In sealed foil bag with desiccant	With proper storage the plate strips are stable until the expiration date.
Enzyme Conjugated Detection Antibody	One vial of 150µL of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8°C in the dark	The 1X working conjugate solution should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date.
Calibrator	One vial of calibrator	Refer to the Certificate of Analysis (CoA).	2-8°C for lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freeze-thaw cycles.	The working standard solutions should be prepared immediately prior to use.
Diluent Concentrate	One 50 mL bottle of 5X diluent buffer	Dilute 1:5 to make 1X working solution.	2-8°C for both 1X working solution and 5X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date.
Wash Solution Concentrate	One 50 mL bottle of 20X wash solution	Dilute 1:20 to make 1X working solution.	2-8°C for both 1X working solution and 20X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date.
Chromogen-Substrate Solution	One bottle of 12 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	Ready to use as supplied	2-8°C in the dark	Protect from light. The Substrate Solution is stable until the expiration date.
STOP Solution <u>WARNING:</u> <u>Avoid</u> <u>Contact with</u> <u>Skin</u>	One 12 mL bottle of 0.3 M sulfuric acid.	Ready to use as supplied	2-8°C	The Stop Solution is stable until the expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µL to 1000 µL) for making and dispensing dilutions
- Test tubes
- Microtiter washer/aspirator
- Distilled or Deionized H₂O
- Microtiter Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Centrifuge (for sample collection)
- Anticoagulant (for plasma sample collection)

SAMPLE COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing. If blood samples are clotted, grossly hemolyzed, lipemic, or if the integrity of the sample is of concern, make a note and interpret results with caution. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- Serum Samples - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Plasma Samples - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.

DILUTION OF SAMPLES

The assay requires that each sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Plasma and Serum Samples – The recommended starting dilution is 1:3000. To prepare a 1:3000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X working diluent. This gives a 1:100 dilution. Next, dilute the 1:100 samples by transferring 10 µL to 290 µL of 1X working diluent. This yields a 1:3000 dilution of the sample. Mix thoroughly at each stage.

REAGENT PREPARATION

- **Bring all reagents to room temperature (16-25°C) before use.**
- Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH₂O).
- Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH₂O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.
- Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X working diluent for each test strip to be used. Mix uniformly, but gently. Avoid foaming.
- Rat Alpha 2-Macroglobulin Calibrator - Prepare according to the lot specific Certificate of Analysis.

ASSAY PROCEDURE

1. All samples and standards should be assayed in duplicate.

2. The standards and the samples should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

Pipette 100 μ L of:

- Standard 0 (0.0 ng/mL) in duplicate
- Standard 1 (6.25 ng/mL) in duplicate
- Standard 2 (12.5 ng/mL) in duplicate
- Standard 3 (25 ng/mL) in duplicate
- Standard 4 (50 ng/mL) in duplicate
- Standard 5 (100 ng/mL) in duplicate
- Standard 6 (200 ng/mL) in duplicate
- Standard 7 (400 ng/mL) in duplicate

3. Pipette 100 μ L of sample (in duplicate) into pre-designated wells.

4. Incubate the microtiter plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately 1X working Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents into a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 μ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 μ L of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100 μ L of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well using a plate reader calibrated to the manufacturer's specifications.

CALCULATION OF RESULTS

1. Subtract the average background value (average absorbance reading of standard zero) from the test values for each well.

2. Average the duplicate readings for each standard and use the results to construct a standard curve. Construct the standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the alpha 2-macroglobulin concentration in original samples.

Appendix A Reference Serum Information

One vial containing a Reference Serum is included with this kit.

Please refer to the enclosed Product Profile Sheet for lot-specific information.

Please note the following:

1. The Reference Serum should be frozen upon receipt. It is stable until the expiry date when stored properly.
2. The Reference Serum should be diluted as appropriate to fit within the standard curve range. Refer to the "Dilution of Samples" section of the protocol for instructions.
3. While pipetting the samples (Procedure section), also pipette the Reference Serum in duplicate.