Package Insert

Rat Alpha 2-Macroglobulin

Catalog Number: R2M21-K01

For Research Use Only. Not for use in diagnostic procedures.

v. 5 (25 OCT 23)

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INTENDED USE

The Eagle Bioscience Alpha 2-Macroglobulin test kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Alpha 2-Macroglobulin in biological samples of rats.
The Alpha-2 Macroglobulin ELISA Assay Kit is for research use only and should not be used for diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY BACKGROUND

Alpha 2-Macroglobulin (A2M) is a major protease inhibitor in serum and an acute phase protein which increases significantly in concentration in the rat as a result of inflammation. The major pathophysiological role for rat alpha 2-macroglobulin has yet to be conclusively defined. This kit is specific to the alpha 2-macroglobulin and will not cross react with the closely related alpha 1-macroglobulin.

PRINCIPLE OF THE ASSAY

In this assay the Alpha 2Macroglobulin present in samples reacts with the anti-Alpha 2-Macroglobulin antibodies which have been adsorbed to the surface of polystyrene microtitre 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 enzyme bound to the immunosorbent 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 A2M 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.

LIMITATION OF 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 instruction 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, and accuracy of reagents with those from other lots or sources

MATERIALS PROVIDED

Component	Description	Preparation	Storage	Stability
ELISA Micro Plate, Antibody coated	One plate of 12 removable 8 well strips, antibody coated	Ready to use	2-8ºC 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 150 µL of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer	Dilute 1/100 immediately prior to use	2-8ºC in the dark	The 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)	2-8ºC for Lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freeze thaw cycles	The working standard solutions should be prepared
Diluent Concentrate	One 50mL bottle of 5X diluent buffer	Dilute 1/5 to make 1X working solution.	2-8ºC for both 1X working solution and 5X Concentrate	The working 1x 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 50mL bottle of 20X wash solution	Dilute 1/20 to make 1X working solution	2-8ºC 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 12mL 3,3',5,5' – Tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	Ready to use	2-8ºC in the dark	Protect from light. The substrate solution is stable until the expiration date
Stop Solution **WARNING: Avoid contact with skin**	One 12mL bottle of 0.3M Sulfuric Acid	Ready to use	2-8ºC	The stop solution is stable until the expiration date

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100µL) for making and dispensing dilutions
- Test Tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

SPECIMEN 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 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 evaluate.

- 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.
- Urine samples - Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. 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 % inhibits the enzyme reaction.

DILUTION OF SAMPLES

The assay requires that each test 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.

- Serum samples - Recommended starting dilution is 1/3,000. To prepare a 1/3,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next dilute the 1/100 by transferring 10µL into 290 µL of 1X diluent. This gives you a 1/3,000 dilution. Mix thoroughly.

- Plasma samples - Recommended starting dilution is 1/3,000. To prepare a 1/3,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next dilute the 1/100 by transferring 10µL into 290 µL of 1X diluent. This gives you a 1/3,000 dilution. Mix thoroughly.

REAGENT PREPARATION

- **Bring all reagents to room temperature (16°C to 25°C) before use.**
- Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with dis-tilled 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 may occur 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 microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1 X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.
- Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
- Rat A2M Calibrator - Prepare according to the lot specific Certificate of Analysis.

ASSAY PROCEDURE

1. All samples and standards should be assayed in duplicates.
2. The Standards and the test sample(s) 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 100µL 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 100µL of sample (in duplicate) into predesignated 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 diluted Wash Solution and aspirate. Repeat three times, for a total of four washed. If washing manually: completely fill

wells with wash buffer, invert the plate then pour/shake out the contents in a waste container.

7. Pipette 100µL 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 5/6
9. Pipette 100µL 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 µL of Stop Solution to each well.
12. Determine the absorbance r(450nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturers specifications.

CALCULATION OF RESULTS

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
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 CRP concentration in original samples.

WARRANTY INFORMATION

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.