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ab157730 alpha 2 Macroglobulin Rat ELISA Kit

For the quantitative measurement of alpha 2 Macroglobulin in Rat biological samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's alpha 2 Macroglobulin Rat ELISA kit is an *in vitro* enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of alpha 2 Macroglobulin in Rat biological samples.

In this assay the alpha 2 Macroglobulin present in samples reacts with the anti- alpha 2 Macroglobulin antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti- alpha 2 Macroglobulin antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound alpha 2 Macroglobulin. Following another washing step, the amount of enzyme bound in complex is measured by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies proportionately with the concentration of alpha 2 Macroglobulin 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 alpha 2 Macroglobulin in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

2. Protocol Summary

Remove appropriate number of antibody coated well strips.

Equilibrate all reagents to room temperature.

Prepare all the reagents, samples, and standards as instructed.

Add standard or sample to each well used. Incubate at room temperature.

Aspirate and wash each well. Add prepared HRP labeled secondary detector antibody. Incubate at room temperature.

Aspirate and wash each well. Add Chromogen Substrate Solution to each well.

Immediately begin recording the color development.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Rat alpha 2 Macroglobulin ELISA Microplate	96 wells	4°C
Rat alpha 2 Macroglobulin Calibrator (lyophilized)	1 vial	4°C
5X Diluent Concentrate	50 mL	4°C
20X Wash Buffer Concentrate	50 mL	4°C
Enzyme-Antibody Conjugate	150 µL	4°C
Chromogen Substrate Solution	12 mL	4°C
Stop Solution	12 mL	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Diluent Solution

The diluent solution is supplied as 5X Diluent Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O). The 1X Diluent Solution is stable for at least one week from the date of preparation and should be stored at 4°C.

9.2 1X Wash Buffer

The wash solution is supplied as 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). 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. The 1X Wash Buffer is stable for at least one week from the date of preparation and can be stored at room temperature (16 - 25°C) or at 4°C.

9.3 1X Enzyme-Antibody Conjugate

Calculate the required amount of 1X Enzyme-Antibody Conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark.

9.4 Chromogen Substrate Solution

Ready to use as supplied.

9.5 Stop Solution

Ready to use as supplied.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Add 1.0 mL of distilled or de-ionized water to the Rat alpha 2 Macroglobulin Calibrator and mix gently until dissolved. The calibrator is now at the concentration stated on the vial.

ΔNote: The reconstituted Rat alpha 2 Macroglobulin Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles).

10.2 Label tube numbers 1 - 7.

10.3 Prepare Standard #1 by adding the appropriate volume of 1X Diluent Solution (see below) to tube #1. Add 140 µL of stock Rat alpha 2 Macroglobulin Calibrator to obtain a concentration at 400 ng/mL and mix thoroughly and gently.

***Example:**

NOTE: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided.

C_S = Starting concentration of reconstituted Rat alpha 2 Macroglobulin Calibrator (variable e.g. 1.811 µg/mL)

C_F = Final concentration of Rat alpha 2 Macroglobulin Calibrator for the assay procedure (400 ng/mL)

V_A = Total volume of stock Rat alpha 2 Macroglobulin Calibrator to dilute (e.g. 140 µL)

V_D = Total volume of 1X Diluent Solution required to dilute stock Rat alpha 2 Macroglobulin Calibrator to prepare **Standard #1**

V_T = Total volume of **Standard #1**

D_F = Dilution factor

Calculate the dilution factor (D_F) between stock calibrator and the **Standard #1** final concentration:

$$Cs/C_F = D_F$$

$$1{,}811 / 400 = 4.53$$

Calculate the final volume V_D required to prepare the **Standard #1** at 400 ng/mL

$$V_A * D_F = V_T$$

$$V_D = V_T - V_A$$

$$140 * 4.53 = 634\ \mu L$$

$$VD = 634 – 140 = 494\ \mu L$$

To tube #1, add 140 µL of reconstituted Rat alpha 2 Macroglobulin Calibrator to 494 µL of 1X Diluent Solution to obtain a concentration at 400 ng/mL (**Standard #1**).

10.4 Add 250 µL 1X Diluent Solution into tube numbers 2 - 7.

10.5 Prepare **Standard #2** by adding 250 µL Standard #1 to tube #2. Mix thoroughly and gently.

10.6 Prepare **Standard #3** by adding 250 µL from **Standard #2** to #3. Mix thoroughly and gently.

10.7 Using the table below as a guide to prepare further serial dilutions.

10.8 1X Diluent Solution serves as the zero standard (0 ng/mL).

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See step 10.3				400
2	250	250	500	400	200
3	250	250	500	200	100
4	250	250	500	100	50
5	250	250	500	50	25
6	250	250	500	25	12.5
7	250	250	500	12.5	6.25

11. Sample Collection and Storage

11.1 Serum:

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation.

11.2 Plasma:

For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results.

Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Precautions**

  For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

- **Additives and Preservatives**

  No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

12. Sample Preparation

General Sample information:

The assay for quantification of alpha 2 Macroglobulin in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/3,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/3,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution.
- Next, dilute the 1/100 samples by transferring 10 µL, to 290 µL of 1X diluent. You now have a 1/3,000 dilution of your sample.
- Mix thoroughly at each stage.

13. Plate preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

14. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

14.1 Pipette 100 µL of each standard, including zero control, in duplicate, into pre designated wells.

14.2 Pipette 100 µL of sample (in duplicate) into pre designated wells.

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

14.4 Following incubation, aspirate the contents of the wells.

14.5 Completely fill each well with appropriately diluted 1X Wash Buffer 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 in a waste container. Follow this by gently striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

14.6 Pipette 100 µL of appropriately 1X 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.

14.7 Wash and blot the wells as described in 14.4 - 14.5.

14.8 Pipette 100 µL of TMB Substrate Solution into each well.

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

14.10 After ten minutes, add 100 µL of Stop Solution to each well.

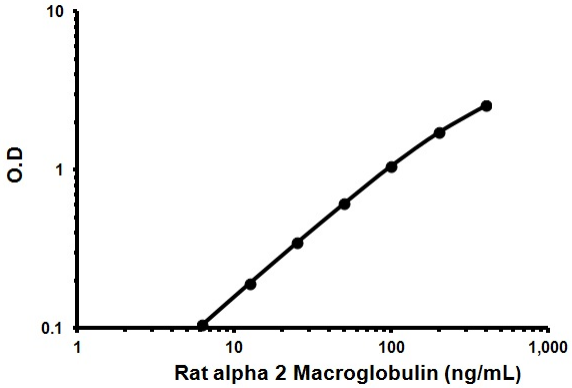
14.11 Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

15. Calculations

Average the duplicate standard reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation Buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

16. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	Mean O.D.
6.25	0.105
12.5	0.192
25	0.346
50	0.612
100	1.059
200	1.717
400	2.541

Figure 1. Example of alpha 2 macroglobulin standard curve. Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

17. Typical Sample Values

SENSITIVITY –

Calculated minimum detectable dose =2.2277 ng/mL

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	< 10%	< 10%

RECOVERY –

Control Serum Recovery = > 85%

18. Interferences

These chemicals or biologicals will cause interferences in this assay causing compromised results or complete failure.

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

20. Notes

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