Human Alpha-2-Macroglobulin Like Protein 1 (a2ML1) ELISA Kit

Cat #: orb2811593 (manual)

Size: 96 tests / 48 tests

For research use only, not for clinical diagnosis.

a2ML1 Introduction

Alpha-2-macroglobulin-like 1, abbreviated as a2ML1, is a protein encoded by the A2ML1 gene. The primary function of A2ML1 is to serve as a broad-spectrum protease inhibitor. It can inhibit all four types of proteases (serine proteases, cysteine proteases, aspartic acid proteases, and metalloproteases) through a unique "trapping" mechanism.

Detection Principle

This kit is based on double antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, biotinylated detection antibodies, and HRP enzyme conjugates were added to the microwells pre-coated with human a2ML1 capture antibody, incubated and washed, and then developed using substrate TMB. TMB is converted to blue under the catalysis of peroxidase (HRP) and then transformed into the final yellow under acidic conditions. The shade of colour was positively correlated with the human a2ML1 in the sample. Measure the absorbance (OD value) with a microplate reader at a wavelength of 450 nm and calculate the sample concentration.

Detection Range: 0.78-50ng/mL

Sensitivity: 0.36ng/mL

Specificity: detects human a2ML1 in samples with no significant cross-reactivity with its analogues.

Product Composition

Reagents	Specifications（96T）	Specifications（48T）	Storage Conditions
Pre-coated Assay Plate	8×12	8×6	2-8°C
Standard	2 tubes	1 tube	2-8°C
Universal Diluent	2×20 mL	1×20 mL	2-8°C
Biotin-antibody (100×)	120 µl	60 µl	2-8°C
Streptavidin-HRP (100×)	120 µl	60 µl	2-8°C
Wash Buffer (20×)	2×10 mL	1×10 mL	2-8°C

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TMB Substrate	10 ml	5 ml	2-8°C
Stop Solution	6 ml	3 ml	2-8°C
Plate Sealer	4 pieces	4 pieces	
Manual	1 copy	1 copy	

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision pipette with disposable tips: 0.5-10uL, 5-50uL, 20-200uL, 200-1000uL
3. 37°C incubator
4. Deionized or distilled water

Precautions

1. Incubation is carried out in strict accordance with the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Refrigerate reagents immediately after use.
2. Incorrect plate washing may result in inaccurate results. Make sure to suction out as much liquid as possible from the wells before adding the substrate. Do not allow the micropores to dry out during the incubation process.
3. Eliminate residual liquid and finger marks from the bottom of the plate, otherwise the OD value will be affected.
4. The substrate chromogenic solution should be colourless or very light in colour, and the substrate solution that has turned blue cannot be used.
5. Avoid cross-contamination of reagents and specimens to avoid false results.
6. Avoid direct exposure to strong light during storage and incubation.
7. No reaction reagent should come into contact with the bleaching solvent or the strong gases emitted by the bleaching solvent. Any bleaching component will destroy the biological activity of the reaction reagent in the kit.
8. Expired products cannot be used, and components with different article numbers and batch numbers should not be mixed.
9. Recombinant proteins from sources other than the kit may not match the antibody of this kit and may not be recognized.
10. If there is a possibility of disease transmission, all samples should be managed, and samples and testing devices should be handled in accordance with the prescribed procedures.

Sample Collection and Requirements

1. **The detection range of the kit is not equivalent to the concentration range of the analyte in the sample,** and it is recommended to estimate the concentration of the analyte in the sample through relevant literature before the experiment and determine the actual concentration of the sample through the pre-

experiment Condition. If the concentration of analyte in the sample is too high or too low, dilute or concentrate the sample appropriately.

2. If the sample to be tested is not among the samples listed in the manual, it is recommended to do a pre-test to verify the validity of the test.

3. **Serum:** Whole blood specimens collected in serum separators are stored at room temperature for 2 hours or 2-8°C overnight, then centrifuge at 1000×g for 20 minutes, and the supernatant can be taken or stored at -20°C or -80°C, but repeated freeze-thaw should be avoided.

4. **Plasma:** Collect specimens with EDTA or heparin as anticoagulants and centrifuge the specimen at 1000×g at 2-8°C for 15 minutes within 30 minutes of collection, take the supernatant for detection, or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.

5. **Tissue homogenate:** Rinse the tissue with pre- cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate can affect the measurement), and mince the tissue after weighing. The minced tissue is compared to the corresponding volume of PBS (generally according to the weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS, the specific volume can be adjusted appropriately according to the needs of the experiment, and a record should be made. It is recommended to add protease inhibitors to PBS) to a glass homogenizer and grind well on ice. To further lyse the histiocytes, the homogenate can be sonicated, or repeatedly freeze-thaw. Finally, the homogenate was centrifuged at 5000×g for 5~10 minutes, and the supernatant was taken for detection.

6. **Cell culture supernatant:** Centrifuge at 1000×g for 20 minutes to detect, or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.

7. **Cell lysate:** For adherent cells, gently wash with pre-cooled PBS, then digest with trypsin, and collect the cells by centrifuging at 1000×g for 5 minutes; suspended cells can be collected directly by centrifugation. Wash the collected cells with pre-cooled PBS 3 times, and resuspend 150-200μL PBS for every 1×10^6 cells (it is recommended to add a protease inhibitor in the PBS; if the content is very low, you can appropriately reduce the volume of PBS) and lyse the cells by repeated freeze-thaw cycles or sonication. Centrifuge the extracted solution at 1500×g for 10 minutes at 2-8°C, and collect the supernatant for detection.

8. **Other biological specimens:** Centrifuge at 1000×g for 20 minutes, and take the supernatant for detection.

9. **Appearance of the sample:** The sample should be clear and transparent, and the suspended solids should be centrifuged and removed.

10. **Sample preservation:** If the sample is tested within 1 week after collection, it can be stored at 4°C, if it cannot be tested in time, please divide it into one-time use and freeze it at -20°C (test within 1 month), or -80°C (testing within 6 months), avoid repeated freezing and thawing, haemolysis of the specimen will affect the final test result, so haemolyzed specimens should not be tested for this test.

Sample Dilution Protocol

Please estimate the concentration range of the samples in advance. If your test sample needs to be diluted, refer to the dilution protocols below.

Dilution 100 times: One-step dilution. Take 5μL of the sample and add it to 495μL of universal diluent to make a 100-fold dilution;

Dilution 1000 times: Two-step dilution. Take 5μL of the sample and add it to 95μL of universal diluent to make a 20-fold dilution, then take 5μL of the 20-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, resulting in a total dilution of 1000 times;

Dilution 100000 times: Three-step dilution. Take 5μL of the sample and add it to 195μL of universal diluent to make a 40-fold dilution, then take 5μL of the 40-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, and finally take 5μL of the 2000-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, resulting in a total dilution of 100000 times;

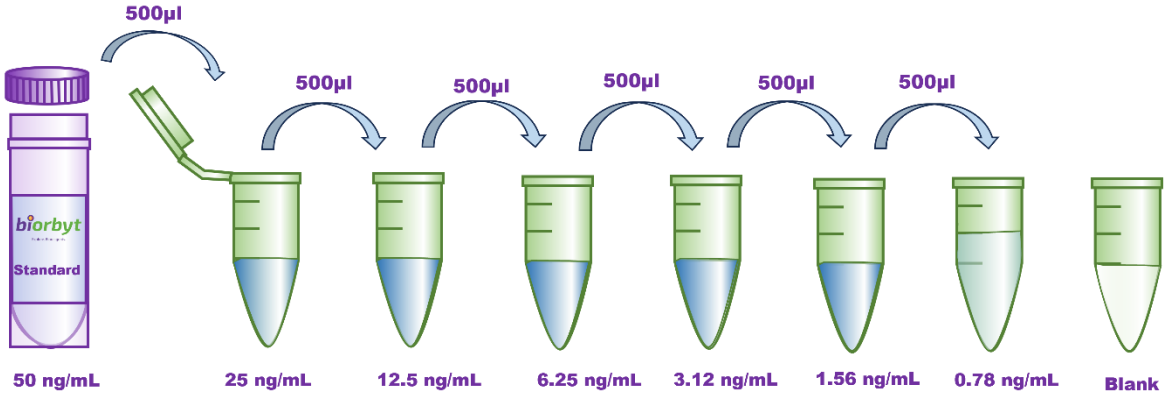
For each dilution step, the volume taken should be no less than 3μL, and the dilution factor should not exceed 100 times. Each dilution step must be mixed thoroughly to avoid foaming.

Reagent Preparation

1. Remove the kit from the refrigerator 10 minutes in advance and equilibrate to room temperature.

2. **Standard gradient working solution:** Add 1mL of universal dilution to the lyophilized standard, let it stand for 15 minutes, let it completely dissolve, and then mix gently (at a concentration of 50 ng/mL), then dilute at the following concentrations: 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL, 1.56ng/mL, 0.78ng/mL, 0ng/mL.

Doubling dilution method: take 7 EP tubes, add 500μL of general dilution solution to each tube, and draw 500μL from 50 ng/mL standard working solution to the first EP tube to mix well to prepare 25 ng/mL standard working solution, and then pipette and mix well according to this step. The last tube is used directly as a blank well, and there is no need to draw liquid from the penultimate tube, as shown in the figure below.



3. **Preparation of biotinylated antibody detection solution:** 15 minutes before use, centrifuge the concentrated biotinylated antibody at 1000×g for 1 minute, and dilute the 100× concentrated biotinylated antibody into a 1× working concentration (10 μL concentrated + 990 μL universal diluent) in a universal diluent, and use it on the same day.

4. **Preparation of enzyme conjugate working solution:** 15 minutes before use, centrifuge 100× concentrated streptavidin-HRP at 1000×g for 1 minute, and dilute 100× concentrated streptavidin-HRP to 1× working concentration in a universal diluent (10 μL concentrated + 990 μL universal diluent) for use on the same day.

5. **Preparation of 1× washing solution:** take 10ml of 20× washing solution to 190ml of distilled water (the concentrated washing solution taken out of the refrigerator may have crystallization, which is a normal phenomenon, it can be placed at room temperature, shake evenly, and then configure after the crystallization is completely dissolved).

Operation Steps

1. Remove the desired slats from the foil pouch after 10 minutes of room temperature equilibration, and place the remaining slats back at 4°C with a ziplock bag.

2. Loading: Add 100 µl of samples or standards of different concentrations to the corresponding wells, and add 100 µL of universal diluent to the blank wells. Incubate for 1 h at 37°C after covering with the plate sealer. (Suggestion: Samples to be tested are diluted by a minimum of 1-fold with a universal diluent before being added to the microplate for testing to reduce the impact of matrix effects on the test results, and the sample concentration should be multiplied by the corresponding dilution factor when calculating the sample concentration. All samples and standards to be tested are recommended to be double-well).

3. Add biotin-antibody: Remove the plate and discard the liquid without washing. 100µL of biotin-antibody working solution was directly added to each well, and incubated at 37°C for 1 hour after covering with a plate sealer.

4. Wash the plate: Discard the liquid, add 300µL of 1x wash solution to each well, let it stand for 1 minute, shake off the wash solution, pat dry on absorbent paper, and repeat the wash 3 times (you can also use a plate washer to wash the plate).

5. Add streptavidin-HRP working solution: Add 100µL of streptavidin-HRP working solution to each well, cover the plate and incubate at 37°C for 30 minutes.

6. Wash the plate: Discard the liquid and wash the plate 5 times according to the washing method in step 4.

7. Add TMB Substrate: 90 µL of TMB Substrate was added to each well, covered with a plate sealer, and incubated at 37°C for 15 minutes in the dark.

8. Add stop solution: Remove the microplate, add 50 µL of stop solution directly to each well, and immediately measure the OD value of each well at a wavelength of 450 nm.

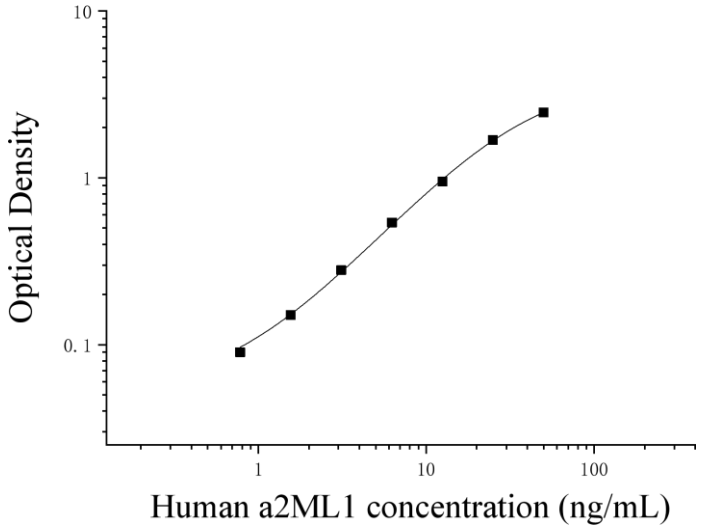
Result Judgment

1. Calculate the average OD value of the standard and sample replicate wells and subtract the OD value of the blank well as the correction value. Using the concentration as the abscissa and the OD value as the ordinate, the standard curve of the four-parameter logistic function is drawn on a double-logarithmic coordinate paper (the value of the blank group is removed when plotting).

2. If the OD value of the sample is higher than the upper limit of the standard curve, it should be diluted appropriately and remeasured and multiplied by the corresponding dilution factor when calculating the sample concentration.

The following data and standard curves are for reference only, and experimenters need to establish standard curves according to their own experiments.

Concentration（ng/mL)	50	25	12.5	6.25	3.12	1.56	0.78	0
OD value	2.55	1.78	1.04	0.63	0.37	0.24	0.18	0.09
Adjusted OD value	2.46	1.69	0.95	0.54	0.28	0.15	0.09	-



Note: This figure is for reference only, and the specimen content should be calculated using the standard curve drawn for the same test standard.

Kit Performance

1. Repeatability: The coefficient of variation within the plate is less than 10%, and the coefficient of variation between plates is less than 10%.

2. Recovery: Recovery was calculated by adding 3 different concentration levels of human a2ML1 to selected healthy human serum, plasma, and tissue homogenate.

Sample type	Range	Average recovery
Serum （n=8)	84-103	97
Plasma (n=8)	90-105	100
Tissue homogenate (n=8)	96-113	104

3. Linear dilution: Linearity was assessed by adding high concentrations of human a2ML1 to 4 selected healthy human serum, plasma, and tissue homogenate, respectively, and diluting within the standard curve kinetics.

Dilution range	Recovery （%）	Serum	Plasma	Tissue homogenate
1： 2	Range （%）	92-113	87-113	86-106
	Average recovery （%）	102	101	98
1： 4	Range （%）	99-110	82-104	96-113
	Average recovery （%）	105	96	103

Troubleshooting

If the experimental results are not good, please take photos of the color development results in a timely manner, save the experimental data, keep the used plates and any unused reagents, and then contact our technical support for assistance. You may also refer to the following materials.

Problems	Possible Causes	Solutions
Linear deviation of the standard curve	Standard sample dilution is incorrect.	Ensure that the standard sample is dissolved and diluted according to the recommended method.
	Inaccurate pipetting.	Regularly calibrate the pipette and check the sealing of the tips.
	Evaporation of reaction solution.	Seal the enzyme labelling plate with sealing film.
	Incomplete washing of the plate.	Sufficient washing cycles and adequate washing liquid should be added.
	Impurities at the bottom of the wells.	Clean the bottom of the plate before reading.
Weak signal or No signal	Insufficient incubation time.	Ensure sufficient incubation time.
	Incubation temperature is incorrect.	Incubate at the recommended temperature.
	Insufficient reagent volume added.	Check the pipette and follow the operational steps rigorously.
	Incorrect dilution.	Check the reagent dilution steps.
	Enzyme conjugate inactivation.	Mix the enzyme conjugate and substrate, and check through the colour reaction.
Low OD	The microplate reader is set up incorrectly.	Check the instrument's wavelength.
	The stopping solution was not added.	Add an appropriate amount of stopping solution.
	The waiting time before reading the plate is too long.	Read the plate in a timely manner.
	The sample concentration is too high.	Determine the appropriate dilution factor through preliminary experiments.
	The sample concentration is too low.	Determine the appropriate dilution factor through preliminary experiments.
High background	The developing solution is contaminated.	Replace the developing solution.
	The developing time is too long.	Control the developing time.
	Incorrect dilution of the antibody	Use the recommended dilution method.

	or enzyme conjugate.	
	Washing of the plates is incomplete.	Ensure enough washing cycles and add sufficient washing solution.

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