Rat α2M ELISA Kit

Cat #: orb1808958 (manual)

Size: 96 tests

This kit is used to quantitatively detect the content of α2-macroglobulin (α2M) in rat serum, plasma, urine, and other biological fluids. Please read the manual carefully and check the reagent components before use. If you have any questions, please contact biorbyt, we will provide you with technical support.

For research use only, not for clinical diagnosis.

Detection Principle

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Purified α2-macroglobulin (α2M) antibody was coated in the microplates, then the standard or the samples to be tested are added to the coated plate wells. After the antigen fully reacts with the coated antibody, the biotinylated α2M antibody and streptavidin labelled with HRP are added in turn. The biotin and streptavidin form high-intensity non-covalent binding. After sufficient washing, the substrate TMB is added for colour development. TMB is converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The shade of colour was positively correlated with the α2M content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of α2M in the sample was calculated by plotting the standard curve.

Product Composition

Reagents	Specifications（96T）	Storage Conditions
Antibody- Coated Slats	8×12	-20 ℃
Standard	2 tubes	-20 ℃
S1 Standard/Sample Dilution Buffer	45 ml×1 bottle	-20 ℃
Detection Buffer A	120μl×1 tube	-20 ℃
Detection Buffer B	120μl×1 tube	-20 ℃
Washing Buffer (Concentrated, 30×)	20ml×1 bottle	2-8℃
TMB Substrate (Avoid direct light)	9ml×1 bottle	2-8℃
Stop Solution	6ml×1 bottle	2-8℃
Plate Sealer	4 pieces	
Manual	1 copy	

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Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision single (0.5-10μL, 2-20μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)
3. Automated plate washer
4. 37°C incubator
5. Deionized or distilled water
6. Coordinate paper
7. Measuring cylinder

Precautions

1. The kit is stored in 2-8℃& -20℃, and the dissolved but unused standard is recommended for disposal. Do not mix kit components from different sources or batch numbers, use this product within the expiration date.
2. When the concentrated Washing Buffer is taken out at low temperature, there may be crystal precipitation, and it can be dissolved by heating in the water bath before dilution, which does not affect the use.
3. Pipettes should be calibrated before and used for each step and to avoid errors. It is recommended to control the sampling time within 5 minutes. If there are a large number of samples, it is recommended to use a multi-channel pipette for sampling.
4. Please make a standard curve at the same time of each determination, and it is better to make a double-check well. If the content of the substance to be tested in the sample is higher than the upper limit of the reagent kit (the OD value of the sample is greater than the OD value of the first well of the standard well), please dilute a certain multiple with the sample dilution buffer before determination. The total dilution multiple shall be multiplied during calculation.
5. In order to avoid cross contamination, remember to replace the tip when adding different concentrations of standard, different samples and different reagents. The plate sealer is only for single use.
6. The TMB substrate should be kept colorless before adding. Do not use the TMB substrate when it turned blue.
7. Strictly follow the manual, and the test results must be based on the microplate reader reading.

Sample Collection and Storage

1. **Serum**: Blood coagulated naturally at room temperature for 60-120 minutes and centrifuged for 20 minutes at 1000 x g. Collect supernatant carefully. If precipitation occurs during storage, centrifuge again to avoid repeated freezing and thawing.
2. **Plasma**: Select EDTA or citric acid as anticoagulant according to sample requirements and centrifuge for about 20 minutes at 1000 x g. Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.
3. **Other biological samples**: When detecting secretory components, collect them with sterile tubes, centrifuge 1000 x g for about 20 minutes, and collect the supernatant.

4. **Urine**: Collect the first urine of the day (mid-section urine) and discharge it directly into a sterile container, centrifuge to remove particulate matter, detect immediately or dispense and store at -20°C; Avoid repeated freeze-thaw.

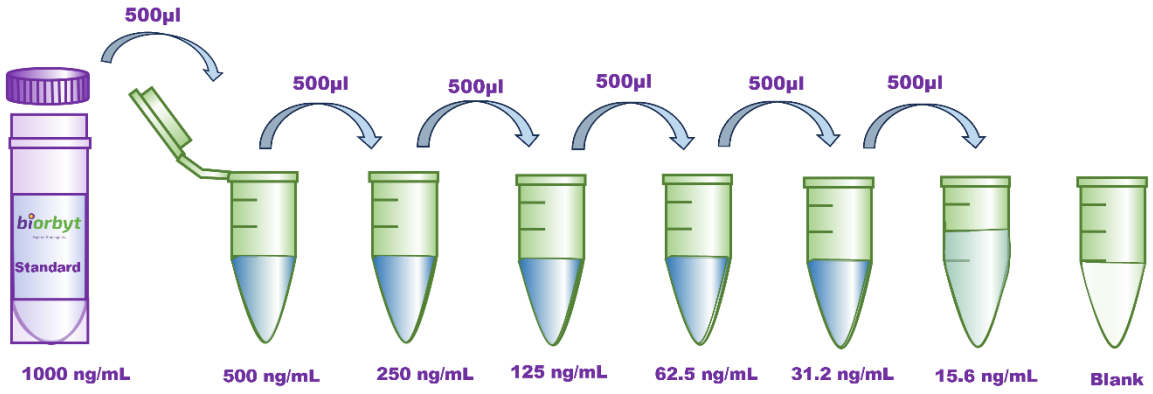
5. If the sample cannot be detected immediately, please divide it into portions according to the minimum usage and store it at -20°C to -70°C to avoid repeated freezing and thawing. Try to avoid using hemolytic or hyperlipid samples.

Reagent Preparation

1. Reagent reheating: Please reheat the reagent kit and the sample to be tested at room temperature within 30 minutes before the test.

2. Preparation of Washing Buffer: Dilute the concentrated Washing Buffer (30×) to Washing Buffer working solution (1×) with double distilled water or deionized water, and keep it as standby.

3. Gradient dilution of standard: Take 1 ml of Standard/Sample Dilution Buffer (S1) into the lyophilized standard, allow it to stand for 15 minutes until it is completely dissolved, then gently mix it with a concentration of 1000 ng/ml, take 6 EP tubes, add 500ul Standard/Sample Dilution Buffer (S1) each EP tube, and dilute twice according to the following concentration: 500, 250, 125, 62.5, 31.2, 15.6 ng/ml were diluted. 1000 ng/ml is the highest concentration of the standard curve, and the Standard/Sample Dilution Buffer (S1) is the zero point (0 ng/ml) of the standard curve. The Standard Stock Solutions (1000 ng/ml) that has not been used up should be discarded.



4. Detection buffer A and Detection buffer B: Please centrifuge it with a few strokes or less before use, so that the liquid of the tube wall or bottle cap is deposited to the bottom of the tube. Before use, dilute 1:100 dilution (e.g., 10µL detection solution A/990µL dilution), mix well, and prepare according to the pre-calculated total amount required for each experiment (100µL/well) before dilution, and 0.1-0.2mL more should be prepared when actually preserved.

Operation Steps

1. Sample addition: According to the required dosage of the test, take out the corresponding antibody-coated slats, and add the prepared standard, standard zero point and sample to the bottom of the experimental well at 100µL/well.

2. Warm incubation: seal the plate with sealing tape and then warm it at 37 °C for 120 minutes.

3. Discard the liquid, shake it dry, and do not wash.

4. Add the working solution of Detection buffer A (prepared before use): add 100 µL of the working solution of Detection buffer A to each well.

5. Warm incubation: seal the plate with sealing tape and then incubate it at 37°C for 60 min.

6. Washing: Carefully remove the sealing tape, discard the liquid, shake dry, fill each hole with washing solution (350µL), let stand for 1-2 minutes and then discard, repeat this 3 times, and finally pat dry on the absorbent paper.

7. Add the working solution of Detection Buffer B (prepared before use): add 100µl of the working solution of Detection Buffer B to each well.

8. Warm incubation: seal the plate with sealing tape and then incubate it at 37°C for 60 min.

9. Washing: Same as the above washing process (step 5), wash the board 5 times.

10. Colour development: Add 90µl of chromogenic substrate solution to each well, seal the plate with sealing tape and set it at 37°C for 15-25min.

11. Termination: Add 50 µL of stop solution to each well (blue turns yellow at this time).

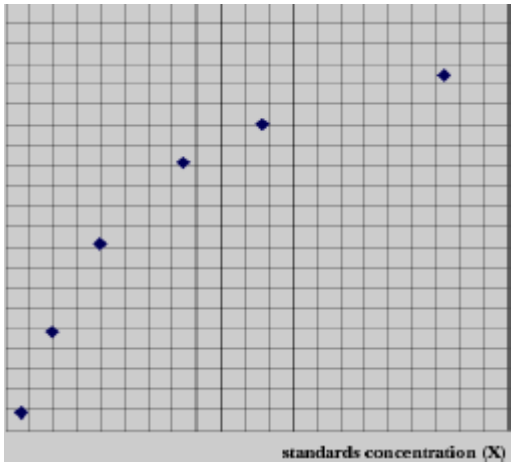
12. Determination: The absorbance (OD value) of each well is measured by the microplate reader at 450nm wavelength, and the measurement should be carried out within 5 minutes after adding the stop solution.

Result Judgment

1. The OD value of each standard and sample minus the OD value of the blank well is the final value, and if a duplicate well is made, its mean value shall be calculated.

2. Take the absorbance OD value as the ordinate (Y) and the corresponding α2M standard concentration as the abscissa (X) to generate the corresponding standard curve. The α2M content of the sample can be calculated from the standard curve according to its OD value. If the OD value of the sample is higher than the upper limit of the standard curve, perform the appropriate dilution during the test, and then multiply the concentration of the sample by the corresponding dilution.

This drawing is for reference only and shall be based on the standard curve drawn in the actual test



Kit Performance

The difference between batches should be less than 10%

Detection Range

15.6 ng/mL -1000 ng/mL

Sensitivity

8.7 ng/ml

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Troubleshooting

Problems	Possible Causes	Solutions
No signal	Mixing Reagents with Different ELISA kits or Batch Numbers	Recheck the label of the reagents to make sure that all components are in the testing kit being used. Do not mix reagents of different testing kits or batch numbers.
	Missing antibody, enzyme and chromogenic agent	Check the operation procedure, and be careful not to omit adding.
	HRP enzyme contaminated with sodium azide	Re-preparation of reagent, no sodium azide
	Wrong reagent preparation/use	Redo the test, operate in strict accordance with the manual, and see the labels clearly before each preparation and use
Weak signal	Reagents Expires expiration date	Check product validity
	Insufficient incubation time	Check the incubation time
	Use of contaminated reagents	Check if reagent is contaminated
	Incorrect instrument setting, filter mismatch	Whether the instrument is set correctly and the filter is used, etc.
	Washing operation is not standard	If the washing is insufficient, increase the number of washing times or extend the washing time
		Wash the bottle, each well shall be completely filled with washing buffer, and pour out quickly
		If a plate washer is used, it shall be calibrated and set to a volume sufficient to fill each hole and the inside of the plate shall not touch the equipment
		Check whether there is residual washing liquid in each well or the volume of sample added in each well is accurate
		You can add a 30 second soak between washings
High background	Improper incubation temperature and time in the experiment	Determine the appropriate incubation temperature and time for each test step
	Excessive enzyme addition	Check whether the regulating amount of pipette is correct before adding enzyme
		Check dilution and perform titer determination if necessary
The standard curve is good, but the sample wells have no signal	Low content of target in sample or no target in sample	Set the positive control and repeat the experiment
	Sample matrix effect influence detection	Test again after re-diluting sample
The standard curve is good, but the sample wells have high signal	The content of sample to be tested exceeds the standard curve range	Test again after re-diluting sample