Mouse Antiserum Titer Test ELISA Kit

(Do not mix reagents of different batches and different product numbers in the kit, otherwise the kit will not work properly)

Catalogue No.: EM2083

Size: 96T

Reactivity: Mouse

Application: Mouse antiserum titers used for immunization with a specific antigen .

Storage: 2-8℃

Expiry Date: see kit label

Principle: Indirect

NOTE: FOR RESEARCH USE ONLY.

Kit Components

No.	Item	Specifications(48T/96T)	Storage
E001	ELISA Microplate(Dismountable)	8×6/8×12	2-8°C/-20°C
E051	Protein coating solution	10ml	2-8°C
E052	Blocking buffer	10ml	2-8°C
E039	Sample Dilution Buffer	10ml/20ml	2-8°C
E003	HRP-labeled Antibody(Concentrated)	60ul/120ul	2-8°C(Avoid Direct Light)
E040	Antibody Dilution Buffer	5ml/10ml	2-8°C
E024	TMB Substrate	5ml/10ml	2-8°C(Avoid Direct Light)
E026	Stop Solution	5ml/10ml	2-8°C
E038	Wash Buffer(25X)	15ml/30ml	2-8°C
E006	Plate Sealer	3/5pieces	
E007	Product Description	1copy	

Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology. Microplate provided in kit for coated antigen (customer itself).The HRP conjugated antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, HRP detection antibody was added to wells. After washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of target antigen in the sample is positively correlated with OD450 and can be calculated by plotting the standard curve.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manuf acturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)

2.37℃ incubator

3.Automated plate washer

4.Precision single and multi-channel pipette and disposable tips

5.Clean tubes and Eppendorf tubes

6.Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

Note: Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

1. Wash Buffer

Dilute 30ml (15ml for 48T) concentrated wash buffer to 750ml (375ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8℃.

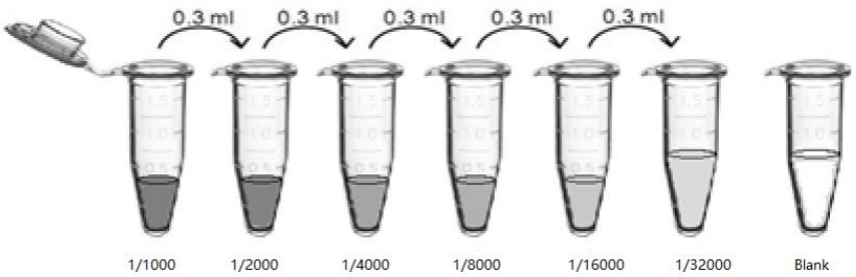
Crystals formed in the concentrated wash buffer can be heated by water bath at 40℃ till complete dissolution. (Heating temperature should be below 50℃.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8℃ within 48h.

2. Gradient dilution of antiserum samples

2.1. Add 10ul of raw antiserum to an EP tube with 990 ml of sample dilution (labeled 1 / 100 tubes) and then mix thoroughly.

2.2.Add 60ul of sample from 1 / 100 tubes to EP tube with 540ul sample dilution (labeled 1 / 1000 tube) and mix thoroughly

2.3.Six additional EP tubes were taken and labeled as 1 / 2000,1 / 4000,1 / 8000,1 / 16000,1 / 32000, and blank. 0.3ml of the sample dilution was added to each EP tube. Add 0.3 ml of the sample solution in 1 / 1000 tubes to the first EP tube and mix thoroughly. Then, 0.3ml of the mixed sample solution in the first EP tube was transferred to the second EP tube and fully mixed. Transfer 0.3ml of the mixed standard solution in the second tube to the third tube, mix well, the rest may be deduced by analogy. Blank EP Only the sample dilutions were available in the tube.



Prepare antiserum gradient dilution solution

Note: The diluted sample solution should preferably be tested within 2 hours.

3. Preparation of HRP-labeled Antibody Working Solution

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

3.1. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

3.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated HRP-labeled antibody to the bottom of tube.

3.3. Dilute the HRP detection antibody with antibody dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated HRP-labeled antibody into 990ul antibody dilution buffer.)

Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely.

1. Antigens were dissolved or diluted with protein-coated solution to control antigen concentration of 1 ug/ml and added to microplates with 100ul antigen solution per well, incubated at 4°C overnight or 37°C for 2 hours.

2. Discard the liquid in the well, add the blocking solution at 100ul per well, and incubate for 60 minutes at 37°C.

3. Wash two times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step two times.

4. Set the gradient dilution sample holes and blank holes of the antiserum to be tested, and record their location. To reduce the experimental error, it is recommended that each sample should be measured in duplicate.

5. Add sample: Add 100ul of antiserum to each well, Seal the plate, and incubate for 90 minutes at 37°C.(Add the solution to the bottom of the microplate and avoid contact with the tube wall and foaming as much as possible.）

6. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.

7. HRP-labeled Antibody: Add 100ul HRP-labeled antibody working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C.

8. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 3.

9. TMB Substrate: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.
(Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable. Please refer to TMB color rendering control in page 2 or QR code for detail.)

10. Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

11. OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately.

Note: When the OD450 value of the antiserum is between 1.0 and 1.2, the corresponding dilution ratio is the titer or titer of the antiserum.

Assay Procedure Summary