FineTest®

Mouse monoclonal antibody isotypte ELISA Kit

Catalogue No.: EM1812

Size: 48T/96T

Reactivity: Mouse

Application: For qualitative isotype determination of mouse immunoglobulins from hybridoma cell culture supernatant or purified antibodies.

Storage: 2-8°C

Expiry Date: see kit label

Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(48T/96T)	Storage
Micro ELISA Plate(Dismountable)	8×6/8×12	2-8°C/-20°C
Positive control	1 ml	2-8°C
Negative control	1 ml	2-8°C
Sample/Standard dilution buffer	10ml/20ml	2-8°C
Biotin-Anti- mouse IgG1, IgG2a, IgG2b, IgG3, and IgM antibodies (Concentrated)	30ul/60ul	2-8°C(Avoid Direct Light)
Antibody dilution buffer	5ml/10ml	2-8°C
HRP-Streptavidin Conjugate(SABC)	60ul/120ul	2-8°C(Avoid Direct Light)
SABC dilution buffer	5ml/10ml	2-8°C
TMB substrate	5ml/10ml	2-8°C(Avoid Direct Light)
Stop solution	5ml/10ml	2-8°C
Wash buffer (25X)	15ml/30ml	2-8°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- mouse IgG antibody was pre-coated onto 96-well plates. And the biotin conjugated Anti- mouse IgG1, IgG2a, IgG2b, IgG3, and IgM antibodies were used as detection antibodies. The positive control, test samples and biotin conjugated detection antibodies were added to the wells, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader.

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.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don’t let Micro plate 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 manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is nece ssary.

Material Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C 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 350ulwash 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, wash plate 3 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°C for long term. Avoid multiple freeze-thaw cycles.

- Hybridoma cell culture supernatant: Collect the clear supernate and carry out the assay immediately.
- Purified antibodies: Samples should be clear and transparent and be centrifuged to remove suspended solids. Before testing, it is necessary to dilute the purified antibody to 10ng/ml.

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

1, Wash Buffer:

Dilute 30ml of Concentrated Wash Buffer to 750 ml of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Preparation of Positive control working solution:

Prepare within 1 hour before the experiment.

1).The positive control contain mouse IgG1, IgG2a, IgG2b, IgG3, and IgM Isotype, and have a positive reaction with Biotin-Anti-mouse IgG1, IgG2a, IgG2b, IgG3, and IgM Isotype antibodies respectively.

2).Dilute the Positive control with Sample/Standard dilution buffer at 1:10 and mix thoroughly.(i.e. Add 100µl of Positive control into 900µl of Sample dilution buffer.)

3, Preparation of Biotin-detection Antibody working solution

Prepare within 1 hour before the experiment.

1) Calculate the total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1µl of Biotin-detection antibody into 99µl of Antibody dilution buffer.)

4, Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30min before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of SABC into 99 µl of SABC dilution buffer.)

Assay Procedure

Before adding to wells, equilibrate the SABC working solution and TMB substrate for at least 30 min at 37°C. When diluting samples and reagents, they must be mixed completely and evenly.

1. Set Positive, negative control and test samples wells on the pre-coated plate respectively. **One test** sample need 5 wells, 5 positive and 5 negative control wells. If need to detect one sample A, you need 1×5 (remark number as A1,A2,A3,A4,A5)+10=15wells. If need to detect two samples, you need 2×5+10=20 wells, and so on. And then, record their positions. The sample position sequence can be arranged as follows:

	sample A	sample B	sample C	sample D	sample E	sample F	negative control	Positive control
Biotin-Anti- mouse IgG1	A1	B1	C1	D1	E1	F1		
Biotin-Anti- mouse IgG2a	A2	B2	C2	D2	E2	F2		
Biotin-Anti- mouse IgG2b	A3	B3	C3	D3	E3	F3		
Biotin-Anti- mouse IgG3	A4	B4	C4	D4	E4	F4		
Biotin-Anti- mouse IgM	A5	B5	C5	D5	E5	F5		

2. Aliquot 100µl of test samples into the test samples wells.
3. Add 100µl of positive and negative control working solution into the control wells.
4. Seal the plate with a cover and incubate at 37°C for 90 min.
5. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. **Wash plate 2 times.** Do NOT let the wells completely dry at any time.

6. Add 100µl of biotin labeled antibody working solution to the corresponding wells (e.g., add Biotin-anti-mouse IgG1 antibody to A1,B1,C1 and the corresponding negative and positive control Wells; Add biotin-anti-mouse IgG2A to A2,B2,C2, and corresponding negative and positive control wells, and so on).
7. Seal the plate with a cover and incubate at 37°C for 60 min.
8. Remove the cover, and wash plate 3 times with Wash buffer.
9. Add 100µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
10. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.
11. Add 90µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark within 10-15 min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user.)
12. Add 50µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
13. Read the OD. Absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

Summary

1. Add 100µL positive, negative control and sample to the corresponding wells for 90 minutes at 37°C
2. add 100µL Biotin-detection antibody working solution to the corresponding wells for 60 minutes at 37°C
3. Aspirate and wash 3 times
4. Add 100µL SABC working solution to each well. Incubate for 30 minutes at 37°C
5. Aspirate and wash 5 times
6. Add 90µL TMB substrate. Incubate 10-15 minutes at 37°C
7. Add 50µL Stop Solution. Read at 450nm immediately

Typical Data & Standard Curve

Results of a Mouse monoclonal antibody isotypte ELISA Kit are shown below. This data was generated at our lab for demonstration purpose only. Each user should obtain their own data as per experiment. (N/A=not applicable)

	Sample A (OD450)	Sample B (OD450)	Sample C (OD450)	Sample D (OD450)	Sample E (OD450)	Sample F (OD450)	Negative control	Positive control
Biotin-Anti- mouse IgG1	A1 (2.78)	B1 (0.252)	C1 (0.126)	D1 (0.183)	E1 (0.210)	F1 (0.226)	0.189	2.639

Biotin-Anti- mouse IgG2a	A2(0.122)	B2 (2.011)	C2 (0.053)	D2 (0.086)	E2 (0.074)	F2 (0.054)	0.089	2.586
Biotin-Anti- mouse IgG2b	A3(0.065)	B3 (0.048)	C3 (1.507)	D3 (0.074)	E3 (0.025)	F3 (0.065)	0.075	2.515
Biotin-Anti- mouse IgG3	A5(0.156)	B5 (0.120)	C5 (0.110)	D5 (0.102)	E5 (1.854)	F5 (0.020)	0.095	2.911
Biotin-Anti- mouse IgM	A6(0.075)	B6 (0.051)	C6 (0.078)	D6 (0.069)	E6 (0.101)	F6 (2.142)	0.081	2.091

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	2-8°C for 6 months
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.