Technical Manual

Mouse monoclonal antibody isotype ELISA Kit (MOFI01463)

- Catalogue Code: MOFI01463
- Indirect ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

monoclonal

Detection method:

Indirect

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Mouse

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Indirect ELISA kit is a highly sensitive assay for the qualitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

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 are used as detection antibodies. The positive control, test samples and biotin conjugated detection antibodies have to be added to the wells and washed with wash buffer. After HRP-Streptavidin addition, unbound conjugates have to be washed away with wash buffer. TMB substrates are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 12	2-8°C/-20°C
2	Positive Control	1ml x 1	2-8°C
3	Negative Control	1ml x 1	2-8°C
4	Sample/Standard dilution buffer	20ml x 1	2-8°C
5	Biotin-Anti- mouse IgG1, IgG2a, IgG2b, IgG3, and IgM antibodies (Concentrated)	60ul	2-8°C (Avoid Direct Light)
6	Antibody dilution buffer	10ml	2-8°C
7	HRP-Streptavidin Conjugate (SABC)	120ul	2-8°C (Avoid Direct Light)
8	SABC dilution buffer	10ml	2-8°C
9	TMB substrate	10ml	2-8°C (Avoid Direct Light)
10	Stop solution	10ml	2-8°C
11	Wash buffer (25X)	30ml	2-8°C
12	Plate Sealer	5 pieces	
13	Product Description	1 copy	

Additional materials required:

1. 37°C incubator
2. Plate Reader with 450nm filter
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

Precautions:

1. To identify the concentration of your target, a 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, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

5. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolyzed samples are not suitable for use with this kit.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolyzed samples are not suitable for this assay.

6. Standard and Reagent Preparation

Manual Washing

Discard the solution on the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes 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 has 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.)

7. Assay Procedure

1. Label the sample wells, 5 Negative Controls, 5 Positive Controls and 5 blank wells.

2. Add 100μL Negative Controls and Positive Controls to each well (except blank well).

3. Add 100μL sample dilution buffer to sample wells and then add 10μL sample serum or plasma. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 90 min.

4. Remove the cover, and wash plate 2 times with Wash buffer and let the wash buffer stay in the wells for 1- 2 minute each time.

5. 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).

6. Seal the plate with a cover and incubate at 37°C for 60 min.

7. Remove the cover, and wash plate 3 times with Wash buffer.

8. Add 100μl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.

9. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stayin the wells for 1- 2 minute each time.

10. Add 90μl of TMB substrate A and 50μl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in the dark for 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

11. Add 50μl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero).

8. Typical Data & Standard Curve

Results of a Mouse monoclonal antibody isotype ELISA Kit is 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

9. Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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