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Fish Immunoglobulin M (IgM) Elisa kit

Product Number: ELK11700

Shipping and Storage

1. Each component of the kit shall be stored at 2~8℃.
2. Ensure the kit has been stored under specified conditions before use. Prepare only the required volume of reagents for each assay.
3. Preparation of 1×Wash Solution: If crystals precipitate in the concentrated wash solution (20×), heat it at 37℃ until all crystals dissolve before use. Dilute with distilled water at a ratio of 1:20. For example, add 10mL of concentrated wash solution to 190mL of distilled water and mix thoroughly. The prepared wash solution can be stored at room temperature for 1 week.

Component

Label	Kit Components	48T	96T
Microplate	Coated Microplate	8×6 strips	8×12 strips
Positive Control	Positive Control	1mL	1mL
Negative Control	Negative Control	1mL	1mL
Enzyme conjugate	HRP-labeled Anti-human IgG	6mL	12mL
Standard diluent	PBS, BSA, etc.	15mL	15mL
20× concentrated wash buffer	20×Concentrated Wash Solution	25mL	25mL
Chromogenic Substrate	Hydrogen Peroxide, TMB	12mL	12mL
Stop solution	Dilute Sulfuric Acid, Anti-precipitation Agent	6mL	6mL

Description

This kit is based on the double-antibody sandwich principle. Capture antibodies are pre-coated on the microplate. After adding samples, the antigen in the sample is captured. HRP-labeled detection antibody is then added to form an "antibody-antigen-enzyme-labeled antibody" sandwich complex. Excess impurities are removed by washing. TMB substrate is added for color development, followed by stop solution. The OD value is measured at 450nm using a microplate reader. The color intensity is proportional to the concentration of the target antigen in the sample.

Application

This kit is used for the detection of fish immunoglobulin M (IgM) in samples such as serum and plasma. It is for research use only and not for diagnostic purposes.

Required but not supplied materials

1. Distilled or deionized water, consumables such as disposable centrifuge tubes and disposable gloves;
2. Pipettes, multi-channel pipettes and matched pipette tips;
3. Containers such as beakers, graduated cylinders and reagent bottles;
4. Plate sealers or alternative materials for sealing the microplate;
5. Auxiliary equipment including microplate shaker (if needed), centrifuge, vortex mixer;
6. Incubator, constant temperature water bath, microplate reader and plate washer.

Specimen requirements

1. The sample collection and storage conditions listed below serve as general guidelines. The storage and operational stability of different sample types have not been fully evaluated.
2. Serum: Collect whole blood using serum collection tubes. Allow whole blood to stand at room temperature for blood cell coagulation, then harvest serum, or centrifuge directly at 1000×g for 15 minutes to obtain serum. Handle samples gently to avoid

hemolysis. Test the obtained serum promptly. If testing cannot be performed immediately, aliquot the serum into multiple portions and store at -20℃ or below for no more than 6 months. Frozen serum samples shall not be subjected to more than 2 freeze-thaw cycles.

3. Plasma: Collect plasma using EDTA or heparin blood collection tubes. Centrifuge the collected whole blood at 1000×g for 15 minutes to harvest plasma. Handle samples gently to avoid hemolysis. Test the obtained plasma promptly. If testing cannot be performed immediately, aliquot the plasma into multiple portions and store at -20℃ or below for no more than 6 months. Frozen plasma samples shall not be subjected to more than 2 freeze-thaw cycles.
4. Samples containing sodium azide, suspended fibrin or aggregates, or severely hemolyzed samples are not suitable for testing.
5. Samples shall be free from microbial contamination.

Protocol

Allow all reagents to equilibrate to room temperature for approximately 30 minutes before use. Alternatively, place them in a 37℃ incubator for rapid warming to room temperature.

Note: Do not open the microplate package until it has reached room temperature.

1. Arrange wells properly for positive controls, negative controls, blank controls and samples.
2. Add 100μL positive control to each positive control well and 100μL negative control to each negative control well. Set up 2 wells for each of the positive and negative controls.
3. For sample wells, add 100μL sample diluent first, followed by 10μL sample.
4. No sample or enzyme is added to blank wells; all other operations are identical to those for sample wells.
5. Cover with plate sealer and incubate at 37℃ for 30 minutes.
6. Wash the plate 5 times. After the final wash, discard residual wash solution, invert the microplate and tap dry on clean absorbent paper.
7. Add 100μL enzyme conjugate working solution to each well.
8. Cover with plate sealer and incubate at 37℃ for 30 minutes.
9. Wash the plate 5 times. After the final wash, discard residual wash solution, invert the microplate and tap dry on clean absorbent paper.
10. Add 100μL chromogenic substrate to each well.
11. Incubate at 37℃ in the dark for 15 minutes.
12. Add 50μL stop solution to each well.
13. Single-wavelength measurement: read absorbance at 450nm using a microplate reader; dual-wavelength measurement: read absorbance at 450/630nm using a microplate reader..

Cut-Off Value for Positivity

Cut-Off = Mean OD value of negative control wells × 2.1 + 0.1 (If the mean OD value of negative control wells is less than 0.050, use 0.050 for calculation).

Example: If the mean OD value of negative control wells is 0.100, the Cut-Off value = 0.100 × 2.1 + 0.1 = 0.310;

If the mean OD value of negative control wells is 0.020, the Cut-Off value = 0.050 × 2.1 + 0.1 = 0.205.

Interpretation of Results

Assay validity criteria: The OD value of positive control > 0.6 and the OD value of negative control < 0.15;

Positive result: If the sample OD value > Cut-Off value, the result is judged positive;

Negative result: If the sample OD value ≤ Cut-Off value, the result is judged negative;

Limitations of the Assay

Accurate interpretation cannot be obtained when the sample concentration is below the limit of detection. This reagent is for qualitative testing only and shall not be used for quantitative measurement.

Product Performance Specifications

The following results are obtained from experiments using calibrator concentration values, and potential influences such as matrix effects and other factors are not included.

Three batches of enterprise positive reference materials were tested, and all results were positive. Three batches of enterprise negative reference materials were tested, and all results were negative. Precision: Intra-assay CV < 10% (N=20), inter-assay CV < 15% (N=20).

Analytical specificity: Other related factors were prepared at 10μg/mL in dilution buffer for measurement, and no obvious cross-reactivity was observed.

Note

1. All components in this kit are for research use only!
2. The stop solution contains dilute sulfuric acid and is corrosive; handle with caution.
3. Kit components contain preservatives, proteins and other ingredients, which may cause skin allergic reactions. Wear a mask to avoid inhaling mist.
4. Chromogenic substrate and concentrated wash solution may irritate the skin, eyes and respiratory tract. Wear a mask to avoid inhaling mist.
5. Wear protective gloves, laboratory coat, eye and face protection. Wash hands thoroughly after experimental operations.

Technical Notes

1. Kits of different batches must not be mixed. It is not recommended to assemble microplate strips from different plates onto one plate for testing. Even for reagents of the same batch, plate-to-plate variation may exist.
2. A standard curve shall be prepared and run in parallel for each assay. The standard curve from a previous assay cannot be used to calculate results for subsequent tests.
3. Do not use kits beyond their expiry date for testing.
4. The chromogenic substrate should be colorless. A blue color indicates deterioration, and such substrate must not be used in experiments.
5. Seal microplate strips with an appropriate plate sealer to ensure reliable test results.
6. Avoid bubble formation during operation. Do not cross-contaminate by swapping caps between different reagent bottles.
7. The assay shall be performed in accordance with the instructions for use. Deviations from the instructions may lead to variable test results. Any operations not following the manual shall be referred to technical support for advice in advance. Otherwise, we do not guarantee the reliability of test results.