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Fish Immunoglobulin Z (IgZ) ELISA kit(Quantitative)

Product Number: ELK11701

Shipping and Storage

1. Reagent kit storage: 2-8℃.
2. Validity period: 6 months.

Component

Label	Kit Components	48T	96T
Microplate	Pre-coated microplate with capture antibody	6 wells×8 strips	12 wells×8 strips
Calibrator (10×)	Analyte (1000μg/mL)	200μL	200μL
Enzyme Conjugate (100×)	100-fold concentrated HRP-labeled detection antibody	100μL	100μL
General Diluent (20×)	Universal diluent for samples / calibrators / enzyme conjugate	15mL	15mL
Concentrated Wash Buffer (20×)	20-fold concentrated wash buffer	25mL	25mL
Chromogenic Reagent	TMB, hydrogen peroxide	12mL	12mL
Stop Solution	Dilute sulfuric acid	6mL	6mL

Description

The kit adopts the double-antibody sandwich principle: capture antibodies are coated on the microplate. After adding the sample, 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 with a microplate reader, and the color intensity is positively correlated with the concentration of the test antigen in the sample.

Application

This kit is used to detect the concentration of fish immunoglobulin Z (IgZ) in samples such as serum and plasma, for research use only.

Testing Scope

1.56-100μg/mL

Required materials not included in the kit

1. Distilled or deionized water, consumables such as disposable centrifuge tubes and disposable gloves;
2. Pipettes, multichannel pipettes and matching tips;
3. Containers such as beakers, graduated cylinders and reagent bottles;
4. Plate sealer film or alternative materials for covering the microplate;
5. Auxiliary equipment including microplate shaker (if needed), centrifuge, vortex mixer;
6. Incubator, water bath, microplate reader, microplate washer.

Reagent Preparation and Storage

1. Preparation of 1×Wash Buffer: If crystals precipitate in the 20×Concentrated Wash Buffer, heat it at 37°C until all crystals dissolve before use. Dilute with distilled water at a ratio of 1:20. Example: add 10mL concentrated wash buffer to 190mL distilled water and mix well.
2. Preparation of 1×Universal Diluent: Dilute with distilled water at a ratio of 1:20. Example: add 10mL of 20×concentrated

universal diluent to 190mL distilled water and mix well.

3. Preparation of 1×Enzyme Conjugate Working Solution: Dilute the 100×enzyme conjugate 1:100 with 1×Universal Diluent. Example: add 10μL of 100×enzyme conjugate to 990μL of 1×Universal Diluent and mix well. The prepared 1×enzyme conjugate working solution can be stored at 2~8°C for 8 hours.
4. Preparation of Working Calibrators: Centrifuge the calibrate vial for 30 seconds before opening to ensure all calibrate is collected at the bottom.
5. Prepare 7 centrifuge tubes. Pipette an appropriate volume of 10× calibrator and dilute it 10-fold with 1× Universal Diluent to obtain calibrator S1. Example: 50μL of 10× calibrator + 450μL of 1× Universal Diluent yields 500μL of S1 calibrator. Add 250μL of 1× Universal Diluent to each of the remaining 6 centrifuge tubes. Serially perform two-fold dilution of S1 into these 6 tubes (S2–S7) for six dilution gradients down to S7. A total of seven calibrators with different concentrations are prepared: S1, S2, S3, S4, S5, S6, S7. 1× Universal Diluent serves as blank calibrator S0.

Sample Collection, Pretreatment and Storage

1. Serum: Collect whole blood with serum collection tubes. Collect serum after blood cells agglutinate at room temperature, or centrifuge directly at 1000×g for 15 minutes to obtain serum. Handle gently to avoid hemolysis. Test the obtained serum promptly. If testing cannot be performed immediately, aliquot the serum, store at −20°C or below for no more than 6 months, and limit freeze-thaw cycles to no more than 2 times.
2. Plasma: Collect plasma using EDTA or heparin blood collection tubes. Centrifuge the collected whole blood at 1000×g for 15 minutes to obtain plasma. Handle gently to avoid hemolysis. Test the obtained plasma promptly. If testing cannot be performed immediately, aliquot the plasma, store at −20°C or below for no more than 6 months, and limit freeze-thaw cycles to no more than 2 times.
3. Tissue: Weigh and mince the tissue. Add lysis buffer to the minced tissue at a weight-to-volume ratio of generally 1:4 to 1:9, e.g., 100 mg tissue sample corresponds to 400μL lysis buffer. The ratio may be adjusted appropriately according to experimental requirements. Centrifuge the homogenate at 5000×g for 5–10 minutes, and collect the supernatant for testing.
4. Cells: Take an appropriate amount of cells, lyse by sonication on ice, centrifuge at 5000×g for 5–10 minutes, and collect the supernatant for testing.
5. Cell culture supernatant: Centrifuge the cell culture supernatant at 5000×g for 5–10 minutes, and collect the supernatant for testing.

Protocol

Allow all reagents to equilibrate to room temperature for approximately 30 minutes before use, or rapidly warm to room temperature in a 37°C incubator.

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

1. Numbering: Calibrator wells, sample wells and blank wells shall be arranged for the assay; layout all samples reasonably.
2. Dilution and loading: Calibrator wells: add 100μL calibrator per well (S1–S7, 7 concentrations in total); Sample wells: add 50μL of 1× Universal Diluent per well, then add 50μL sample; Blank wells: add 100μL of 1× Universal Diluent (S0) per well.
3. Incubation: Cover with plate sealer, incubate at 37°C for 60 minutes.
4. Washing: Wash the plate 3 times. After the final wash, invert the microplate and blot residual liquid on clean absorbent paper.
5. Add enzyme: Calibrator wells / Sample wells: add 100μL of 1× Enzyme Conjugate Working Solution per well; Blank wells: add nothing.
6. Incubation: Cover with plate sealer, incubate at 37°C for 60 minutes.
7. Washing: Wash the plate 5 times. After the final wash, invert the microplate and blot residual liquid on clean absorbent paper.
8. Color development: Add 100μL chromogenic reagent to each well, and develop color at 37°C in the dark for 15 minutes.
9. Stop reaction: Add 50μL stop solution to each well.
10. Measurement: Measure absorbance with a microplate reader at detection wavelength of 450nm (single wavelength); or measure absorbance at 450/630nm (dual wavelength).

11. Note: A preliminary test should be performed to determine the optimal dilution factor.

Calculation

No zero adjustment is required for results obtained by dual-wavelength detection; standard curve fitting and result calculation can be performed directly.

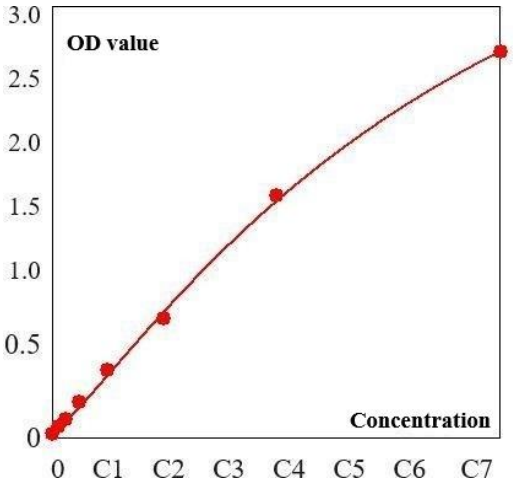
The curve fitting methods listed below are all acceptable. Select the method yielding the best r value for result calculation.

1. 4-parameter logistic (4-P) curve fitting: concentration on the X-axis and absorbance on the Y-axis;
2. Polynomial curve fitting: quadratic polynomial, cubic polynomial;
3. Double-log linear fitting: perform linear fitting after log-transformation of concentration and absorbance values;
4. Point-to-point fitting: separate linear fitting between every pair of adjacent points for segmented calculation.

Professional software is recommended for curve fitting and calculation, such as ELISACALC, SPSS, GraphPad Prism, etc.

The final sample concentration shall be multiplied by the sample dilution factor.

The attached diagram shows curve fitting examples. The data and curves are for illustration only and are unrelated to test results from this kit.



Limitations of the Assay

If the sample concentration exceeds the upper limit of the detection range, the sample shall be diluted at a higher dilution factor and retested. The calculated result should be multiplied by the dilution factor. If the sample concentration is lower than the LOQ (limit of quantitation), accurate quantification cannot be achieved.

Product Performance Specifications

The following results are obtained based on calibrator concentration tests; potential impacts such as matrix effect are not included.

1. Sensitivity: 0.96μg/mL
2. Precision: Intra-assay CV < 10% (N=20), inter-assay CV < 15% (N=20).
3. Analytical specificity: No obvious cross-reactivity was observed when other related factors were tested at 10μg/mL prepared in dilution buffer.

Note

1. All components of 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 and protein ingredients which may cause skin allergic reactions. Wear a mask to avoid inhaling mist.
4. Chromogenic reagent and concentrated wash buffer may irritate the skin, eyes and respiratory tract. Wear a mask to avoid inhaling mist.

5. Wear protective gloves, lab coat, eye and face protection. Wash hands thoroughly after the experiment.

Technical Notes

1. Kits from different batches must not be mixed. It is not recommended to assemble 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 must be prepared and run in parallel for each assay. The standard curve from a previous run cannot be used to calculate results for subsequent assays.
3. Do not use the kit beyond its expiry date.
4. The chromogenic substrate should be colorless. A blue color indicates deterioration, and the substrate must not be used.
5. Use suitable plate sealer film to cover the microplate strips for reliable test results.
6. Avoid bubble formation during operation. Do not cross-contaminate by swapping caps between different reagent vials.
7. Follow the instructions in the manual strictly. Deviation from the protocol may lead to variable test results. Any off-protocol operation should be consulted with after-sales technical support in advance; otherwise, we do not guarantee reliable test results.