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Fish Immunoglobulin Z (IgZ) ELISA kit(Qualitative)

Product Number: ELK11699

Shipping and Storage

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

Component

Component	96T
30× concentrated wash buffer	20mL×1
Enzyme conjugate	6mL×1
Microplate (Coated)	12 wells×8 strips
Sample diluent	6mL×1
Chromogen substrate A	6mL×1
Chromogen substrate B	6mL×1
Stop solution	6mL×1
Positive Control	0.5mL×1
Negative Control	0.5mL×1
Standard diluent	1.5mL×1

Description

This kit adopts the double-antigen sandwich method to detect the expression of fish immunoglobulin Z (IgZ) in specimens. Purified antigen is coated onto microplates to form solid-phase antigen, which can bind to immunoglobulin Z (IgZ) in samples. After washing to remove unbound antibodies and other components, HRP-labeled antigen is added to form an antigen-antibody-enzyme-labeled antigen complex. Following thorough washing, TMB substrate is added for color development. TMB is converted to blue under the catalysis of HRP, and finally turns yellow in the presence of acid. The absorbance (OD value) is measured at 450 nm with a microplate reader and compared with the CUTOFF value to determine the presence or absence of fish immunoglobulin Z (IgZ) in the specimen.

Application

This kit is used for the determination of immunoglobulin Z (IgZ) expression in fish serum, plasma and related liquid samples.

Specimen requirements

1. Extract the specimen as soon as possible after collection, according to relevant literature, and conduct experiments as soon as possible after extraction. If the experiment cannot be conducted immediately, the specimen can be stored at -20℃, but repeated freezing and thawing should be avoided.
2. Samples containing NaN3 cannot be detected because NaN3 inhibits the activity of horseradish peroxidase (HRP).

Protocol

1. Numbering: Label the micro-wells corresponding to samples in sequence. Each plate shall contain 2 wells for negative control, 2 wells for positive control and 1 well for blank control (no sample or enzyme-labeled reagent is added to the blank control well; all other operations remain the same).
2. Sample addition: Add 50μL of negative control and positive control to the negative and positive control wells respectively. For test sample wells, add 40μL of sample diluent first, then add 10μL of test sample. Pipette samples to the bottom of microplate wells, avoid touching the well walls as much as possible, and mix gently.

3. Incubation: Seal the plate with plate sealer and incubate at 37°C for 30 min.
4. Solution preparation: Dilute the 30× concentrated washing solution 30-fold with distilled water for later use.
5. Washing: Carefully remove the plate sealer, discard the liquid and tap dry. Fill each well with washing solution, stand for 30 sec and discard. Repeat 5 times and pat dry.
6. Enzyme addition: Add 50μL of enzyme-labeled reagent to each well except the blank well.
7. Incubation: Follow the same procedure as Step 3.
8. Washing: Follow the same procedure as Step 5.
9. Color development: Add 50μL of Chromogen A followed by 50 μL of Chromogen B to each well, mix gently by shaking, and incubate at 37°C in the dark for 10 min.
10. Termination: Add 50μL stop solution to each well to terminate the reaction (the blue color will immediately turn yellow).
11. Measurement: Zero adjustment with the blank well. Measure the absorbance (OD value) of each well sequentially at 450nm. The measurement shall be completed within 15 min after adding stop solution.

Calculation

Assay Validity: The mean OD value of positive control wells ≥ 1.00; the mean OD value of negative control wells ≤ 0.20.

Calculation of Cut-off Value: Cut-off = mean OD value of negative control wells + 0.15.

Negative Interpretation: Samples with OD value < Cut-off value are negative for fish immunoglobulin Z (IgZ).

Positive Interpretation: Samples with OD value ≥ Cut-off value are positive for fish immunoglobulin Z (IgZ).

Note

1. Strictly follow the manual for operation. Components from different batches of this reagent must not be mixed.
2. After taking the kit out of refrigerated storage, equilibrate it at room temperature for 15–30 minutes before use. If the pre-coated microplate strips are not exhausted after opening, store the strips in a sealed bag.
3. Crystals may precipitate in the concentrated washing solution. Warm it in a water bath to dissolve crystals before dilution; this will not affect the washing results.
4. The plate sealer is for single use only to avoid cross-contamination.
5. Store the substrate away from light.
6. Assay results must be interpreted based on microplate reader readings. For dual-wavelength measurement, the reference wavelength is 630nm.
7. All samples, washing solutions and waste materials shall be treated as infectious substances. The stop solution is 2M sulfuric acid; exercise caution during handling.