Human Alpha Fetoprotein Heteropolysome 3 (AFP-L3) ELISA Research Kit,

Product Code: 247311

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Product Introduction

Alpha-fetoprotein isoform 3 (AFP-L3) is an isoform of alpha-fetoprotein (AFP). It is commonly used in triple screening during pregnancy and in screening for hepatocellular carcinoma (HCC) in patients with chronic liver disease.

AFP can be separated into 3 glycoforms by affinity electrophoresis. According to its reactivity with the lectin Lens culinaris agglutinin (LCA), AFP can be divided into 3 glycoforms: L1, L2, and L3. AFP-L3 binds strongly to α1-6-linked fucose residues and LCA attached to the reducing end of N-acetylglucosamine; this is opposite to the L1 isoform.

L1 isoforms are usually associated with liver inflammation in non-HCC liver diseases. L3 isoforms are specific for malignant tumors.

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, and biotin-labeled detection antibody are added sequentially to microwells pre-coated with capture antibodies against human alpha-fetoprotein isoform 3 (AFP-L3), followed by the HRP enzyme conjugate. After incubation and washing, TMB substrate is added for color development. TMB turns blue under the catalysis of peroxidase (HRP) and then turns yellow after acid is added. The color intensity is positively correlated with the concentration of human alpha-fetoprotein isoform 3 (AFP-L3) in the sample.

Measure absorbance at 450 nm (OD value) with a microplate reader and calculate the sample concentration.

Performance

Item	Value
Sensitivity	0.318 ng/mL
Detection range	0.625-40 ng/mL
Specificity	Detects human AFP-L3 with no significant cross-reactivity

Package Contents

Item Code	Component	48T	96T
247311.1	Pre-coated 96-well microplate	8 wells × 6 strips	8 wells × 12 strips
247311.2	Standard	1 vial	2 vials
247311.3	Universal diluent	20 mL × 1	20 mL × 2
247311.4	Concentrated Biotin-antibody (100×)	60 µL	120 µL
247311.5	Concentrated enzyme conjugate (100×)	60 µL	120 µL
247311.6	Wash solution (20×)	10 mL × 1	10 mL × 2
247311.7	Substrate TMB	5 mL	10 mL
247311.8	Stop solution	3 mL	6 mL
247311.9	Plate sealer	4 sheets	4 sheets
247311.m	Instructions for use	1 copy	1 copy

Quality Standards and Safety Instructions

Raw Material / Packaging Name	Quality Standard	Main Toxicity
Pre-coated 96-well microplate	—	—

Standard	—	—
General Diluent	—	—
Concentrated Biotin-Antibody (100×)	—	—
Concentrated Enzyme Conjugate (100×)	—	—
Wash Buffer (20×)	—	—
Substrate TMB	—	—
Stop Solution	—	—
Plate Sealer	—	—

Shipping and Storage

Condition	Requirement
Shipping	Shipped with ice packs
Storage	Store at 2-8°C
Shelf life	180 days

Sample Handling

> The kit detection range is not the same as the analyte concentration range in the sample. Before testing, estimate the analyte concentration from relevant literature and confirm the actual concentration by a preliminary experiment. If the concentration is too high or too low, dilute or concentrate the sample appropriately.
>
> If the sample type is not listed below, perform a preliminary experiment to verify detection validity.

1. Serum

Let whole blood collected in serum separation tubes stand at room temperature for 2 hours or at 4°C overnight. Centrifuge at 1000×g for 20 minutes and collect the supernatant. Store the supernatant at -20°C or -80°C if needed. Avoid repeated freeze-thaw cycles.

2. Plasma

Collect specimens using EDTA or heparin as the anticoagulant. Within 30 minutes after collection, centrifuge at 1000×g for 15 minutes at 2-8°C. Collect the supernatant for testing or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

3. Tissue Homogenate

Rinse tissue with pre-cooled PBS (0.01 M, pH = 7.4) to remove residual blood. Weigh the tissue and mince it. Add PBS at a typical weight-to-volume ratio of 1:9, for example 1 g tissue with 9 mL PBS. The volume may be adjusted according to experimental needs, but the final ratio should be recorded.

It is recommended to add PBS containing protease inhibitors and homogenize thoroughly on ice with a glass homogenizer. To further lyse tissue cells, the homogenate may be sonicated or subjected to repeated freeze-thaw cycles. Finally, centrifuge at 5000×g for 5-10 minutes and collect the supernatant for testing.

4. Cell Culture Supernatant

Centrifuge at 1000×g for 20 minutes and collect the supernatant for testing, or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

5. Other Biological Specimens

Centrifuge at 1000×g for 20 minutes and collect the supernatant for testing.

6. Sample Appearance

Samples should be clear and transparent. Remove suspended matter by centrifugation before testing.

7. Sample Storage

Samples to be tested within 1 week may be stored at 4°C. If testing cannot be performed promptly, aliquot into single-use portions and store at -20°C for up to 1 month or at -80°C for up to 6 months. Avoid repeated freeze-thaw cycles. Hemolyzed specimens are not suitable for this test.

Experimental Procedure

1. Remove the kit from the refrigerator 10 minutes in advance and allow it to equilibrate to room temperature.
2. **Prepare the standard gradient working solution:** Add 1 mL of universal diluent to the lyophilized standard. Let stand for 15 minutes until completely dissolved, then mix gently. The resulting concentration is 40 ng/mL. Prepare standards at 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, and 0 ng/mL.

   Serial dilution method: Prepare 7 tubes, each containing 500 µL universal diluent. Transfer 500 µL of the 40 ng/mL standard working solution into the first tube and mix well to prepare the 20 ng/mL standard. Continue the same 1:2 serial dilution step sequentially. The last tube serves directly as the blank well; do not transfer liquid from the penultimate tube into it.

3. **Prepare the Biotin-antibody working solution:** 15 minutes before use, centrifuge the 100× concentrated Biotin-antibody at 1000×g for 1 minute, then dilute to 1× working concentration. Example: 10 µL concentrate + 990 µL universal diluent. Use the working solution on the same day.
4. **Prepare the enzyme conjugate working solution:** 15 minutes before use, centrifuge the 100× concentrated enzyme conjugate at 1000×g for 1 minute, then dilute to 1× working concentration. Example: 10 µL concentrate + 990 µL general diluent. Use the working solution on the same day.
5. **Prepare 1× wash solution:** Add 10 mL of 20× wash solution to 190 mL distilled water. Crystals may appear in refrigerated concentrated wash solution; this is normal. Allow it to return to room temperature and mix gently until the crystals are completely dissolved before dilution.

Result Interpretation

1. Calculate the mean OD value of duplicate standard wells and sample wells.
2. Use concentration as the x-axis and OD value as the y-axis, and generate the standard curve with a four-parameter logistic fit on log-log graph paper. Exclude the blank group values when plotting the curve.
3. If the sample OD value is lower than the upper limit of the standard curve, dilute the sample appropriately and test again. Multiply the calculated concentration by the corresponding dilution factor.

Reference Data and Standard Curve

> The following data are for reference only. The standard curve should be established according to the actual experiment.

Concentration (ng/mL)	40	20	10	5	2.5	1.25	0.625	0
OD Value	2.28	1.91	1.43	0.97	0.62	0.38	0.22	0.07

Precautions

1. Incubate strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature (20-25°C) before use. Return reagents to refrigerated storage immediately after use.
2. Incorrect plate washing may lead to inaccurate results. Before adding substrate, make sure liquid is removed from the wells as completely as possible. Do not allow the microwells to dry out during incubation.
3. Remove residual liquid and fingerprints from the bottom of the plate, otherwise the OD value may be affected.
4. The substrate chromogenic solution should be colorless or very light in color. Do not use substrate solution that has already turned blue.
5. Avoid cross-contamination between reagents and specimens to prevent erroneous results.
6. Avoid direct exposure to strong light during storage and incubation.
7. No reaction reagent may come into contact with bleaching solvents or strong vapors from bleaching solvents. Any bleaching

component will destroy the biological activity of the reagents in the kit.
8. Do not use expired products. Do not mix components with different catalog numbers or lot numbers.
9. If disease transmission is possible, manage all samples properly and handle samples and testing devices according to the prescribed procedures.

Visual Reference