Version 08-08/25

User's Manual

Anti-Histone IgG ELISA Kit

REF **DEIA-PY6269**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For quantitative detection of Histone-IgG in serum, plasma, tissue homogenates and other biological fluids.

Principles of Testing

This kit was based on indirect enzyme-linked immune-sorbent assay technology. Antigen was pre-coated onto 96-well plates. And the HRP conjugated antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

Reagents And Materials Provided

ELISA Microplate (Dismountable), 8×12, 2-8°C/-20°C

Lyophilized Standard, 2 vial, 2-8°C/-20°C

Sample Dilution Buffer: 20 ml, 2-8°C

HRP-labeled Antibody (Concentrated): 120 ul, 2-8°C (Avoid Direct Light)

Antibody Dilution: 10 ml, 2-8°C (Avoid Direct Light)

Stop Solution: 10, ml 2-8°C

Wash Buffer(25X): 30 ml, 2-8°C

Plate Sealer: 5 pieces

Product Description: 1 copy

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37°C incubator (CO2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10 μL, 5-50 μL, 20-200 μL, 200-1000 μL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date.

Reagent Preparation

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. **Wash Buffer**

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 been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml (15ml for 48T) Concentrated Wash Buffer to 750ml (375ml for 48T) Wash Buffer with deionized or distilled water (The recommended resistivity of deionized or distilled water is 18MΩ). Put unused solution back at 2-8°C.

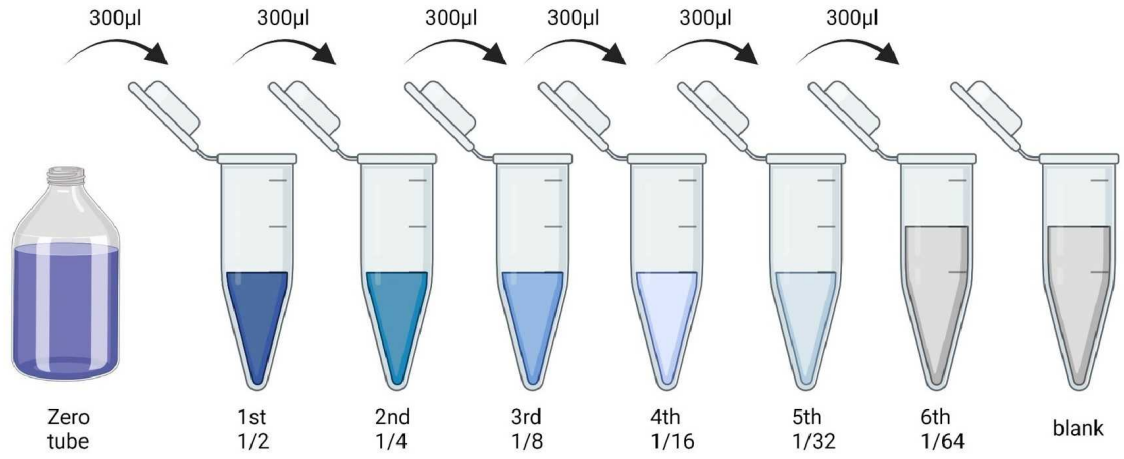
2. **Standards**

2. 1. Centrifuge standards tube for 1min at 10000 xg. Label it as Zero tube.

2. Add 1ml sample dilution buffer into the standard tube. Tighten the tube cap and Let it stand for 2 min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

2. 3. Centrifuge the tubes for 1min at 1000 xg, making the liquid towards the bottom of tube and removing possible bubbles.

2. 4. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3 ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3 ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3 ml sample dilution buffer.



Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

3. **Preparation of HRP-labeled Antibody Working Solution:**

Prepare it within 1 hour before experiment.

1) Calculate required 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 HRP-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 ul HRP-labeled antibody into 99 ul Antibody Dilution Buffer.)

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples (diluted at least 1/100 with Sample Dilution Buffer); control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. Prepare Standards: Aliquot 100ul of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.
3. Add Samples: Add 100ul of properly diluted sample into test sample wells.
4. Incubate: Seal the plate with a cover and incubate at 37°C for 90 minutes.
5. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time.
6. HRP-labeled Antibody: Add 100ul HRP-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 30 minutes.
7. Wash: Remove the cover, and wash plate 5 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.
8. TMB Substrate: Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C.). (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
9. Stop: Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
10. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Assay Procedure Summary

Step 1: Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions.

Step 2: Add 100 µl standard or sample to each well and incubate for 90 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step 3: Add 100 µl HRP-labeled antibody working solution to each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step 4: Add 90 µl TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step5: Add 50 µl Stop Solution. Read at 450nm immediately and calculation.

Detection Range

1.563-100 ng/ml

Detection Limit

0.938 ng/ml

Specificity

Human