Human DS-DNA-IgG (double stranded DNA-Immunoglobulin G) ELISA Kit

Catalogue No.: EH4955

Revision: V 3.0

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of DS-DNA-IgG in serum, plasma, tissue homogenates and other biological fluids.

Storage: 2-8°C

Expiry Date: see kit label

Principle: Indirect

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
ELISA Microplate(Dismountable)	8×12	2-8°C
Negative Control	1.0ml	2-8°C
Positive Control	1vial	2-8°C
Sample Dilution Buffer	20ml	2-8°C
HRP-conjugated anti-human IgG antibody (Concentrated)	120ul	2-8°C
Antibody Dilution Buffer	10ml	2-8°C
Wash Buffer (25 x concentrate)	30ml	2-8°C
TMB Substrate	10ml	2-8°C
Stop solution	10ml	2-8°C
Plate Sealer	3pieces	
Instruction manual	1copy	

Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology. DS-DNA was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP

conjugated anti-human IgG, if there were any DS-DNA-IgG in the samples, it would form a complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650nm.

Precautions for Use

1. 1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)

2. 37°C incubator

3. Automated plate washer

4. Precision single and multi-channel pipette and disposable tips

5. Clean tubes and Eppendorf tubes

6. Deionized or distilled water

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (Note: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- **Serum:** Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000×g for 15 minutes. Analyze the serum immediately or aliquot and store at -20°C.
- **Plasma:** Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 minutes at 2-8°C at 1500xg within 30 minutes of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 minutes at 2-8°C at 1000xg. Analyze immediately or aliquot and store frozen at -20°C.
- **Other Biological Fluids:** Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

The matrix components in the sample will affect the test results, which it need to be diluted at least 1/1000 with Sample Dilution Buffer before testing!

Wash Buffer Preparation:

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 Concentrated Wash Buffer to 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

Preparation of Positive Control

1). Add 1 ml Sample Dilution Buffer into Positive Control tube ，keep the tube at room temperature for 10 minutes and mix them thoroughly.

Preparation of HRP-conjugated anti-human IgG Working Solution:

Prepare it within 1 hour before experiment.

1. Calculate required total volume of the working solution: 100ul/well × quantity of wells. (Allow 100-200ul more than the total volume.)

2. Dilute the HRP-conjugated anti-human IgG with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul HRP-conjugated anti-human IgG into 99ul 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.

1. Bring all reagents to room temperature before use.

2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well.

3. Add 100ul diluted samples to sample wells.

4.Add 100ul Negative Controls and Positive Controls to set Controls well and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 30 minutes.

5. Remove the cover, and wash plate 3 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.

6. Add 100ul HRP-conjugated anti-human IgG Working Solution to each well.

7. Seal the plate with a cover and incubate at 37°C for 30 minutes.

8. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.

9. Add 90ul of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark for 15 minutes. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color. **(Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C.)**

10. Add 50ul of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

11. Read the O.D. absorbance at 450nm in a microplate reader immediately after adding the stop solution (Use the blank well to set zero).

Data Analysis

Calculation of Results

Cutoff Value =NCx + 0.2

NCx: Mean Absorbance of Negative Control (when NCx<0.05, Calculate as 0.05).

PCx: Mean Absorbance of Positive Control

1. Sample with absorbance values < Cutoff Value are considered negative.

   Sample with absorbance value ≥ Cutoff Value are considered positive.

2. PCx≤0.5, the test is regarded as invalid, should be tested again.