# Human AS-IgG (anti sperm-Immunoglobulin G) ELISA Kit

**Catalogue No.:** EH4417

**Size:** 48T/96T

**Reactivity:** Human

**Application:** This immunoassay kit allows for the qualitative determination of AS-IgG in human serum or plasma.

**Storage:** 2-8℃

**Expiry Date:** see kit label

**Principle:** Indirect

**NOTE: FOR RESEARCH USE ONLY.**

**Kit Components**

| Item | Specifications(48T/96T) | Storage |
| --- | --- | --- |
| Micro ELISA Plate(Dismountable) | 8×6/8×12 | 2-8℃/-20℃ |
| AS-IgG Positive Control | 500ul×1/1ml×1 | 2-8℃ |
| AS-IgG negative Control | 500ul×1/1ml×1 | 2-8℃ |
| sample dilution buffer | 10ml/20ml | 2-8℃ |
| HRP-labeled Antibody(Concentrated) | 60ul/120ul | 2-8°C(Avoid Direct Light) |
| Antibody Dilution Buffer | 5ml/10ml | 2-8°C |
| TMB Substrate | 5ml/10ml | 2-8℃(Avoid Direct Light) |
| Stop Solution | 5ml/10ml | 2-8°C |
| Wash buffer (25X) | 15ml/30ml | 2-8℃ |
| Plate Sealer | 3/5pieces | |
| Product Description | 1 copy | |

# Operation Procedure

## Principle of the Assay

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.

## Precautions

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 (universal)

- **Serum:** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

- **Plasma:** Collect plasma using EDTA-$Na_2$ or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

**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.

## Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

**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, Preparation of HRP-labeled Antibody Working Solution:**

Prepare it within 30minutes before experiment.

1 ）Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

2 ）Dilute the HRP-labeled Antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul HRP-labeled Antibody 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. It is recommended to plot a standard curve for each test. .

1. Bring all reagents to room temperature before use.

2. Label the sample wells, 3 Negative Controls, 1 Positive Controls and 1 blank wells.

3. Add 100µL Negative Controls and Positive Controls to each wells(except blank well).

4. Add 50µL sample dilution buffer to sample wells and then add 50µL sample serum or plasma. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37℃ for 30 min.

5. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 0.5-1 minute each time.

6. Add 100µL HRP-labeled Antibody to each well, except blank well

7. Seal the plate with a cover and incubate at 37℃ for 30 min.

8. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 0.5-1 minute each time.

9. Add 50ul of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 10-15minutes. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.

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

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

**Data Analysis**

- **1. Calculation of the Cutoff Value**

  Cutoff Value = **NCx**+0.1

NCx: Mean Absorbance of Negative Control(When the negative mean A value is less than 0.05, it is calculated as 0.05.When the negative mean A value is greater than or equal to 0.05, it is calculated according to the actual value.)

- 2. **Determination of results**

Sample with absorbance values <Cutoff Value are NON-REACTIVE and are considered NEGATIVE for AS-IgG. Sample with absorbance values ≥ Cutoff Value are considered POSITIVE for AS-IgG.

- 3. **Quality control**

The blank well (only adding TMB and Stop solution) should not be greater than 0.08.

The positive control (PC) A value was greater than 0.50.

The negative mean A value was less than 0.08.