



## Technical Manual

### Human Anti-Ovary (AO) IgG ELISA Kit

- Catalogue Code: HDES0078
- Antibody ELISA Kit
- Research Use Only

## 1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the anti-Ovary (AO) antibody (IgG) in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified ovary membrane antigen. Samples are added to the ELISA Microtiter plate wells and the AO-Ab in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugate is added to each well and react with the compound to form “ovary membrane antigen- AO antibody-HRP conjugate” compound. The Substrate Reagent is added to initiate the color developing reaction. The presence of AO-Ab (IgG) can be determined according to the OD value after colorimetric assay with the Micro-plate reader.

## 2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

## 3. Experimental instrument

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper
- Loading slot for Wash Buffer

## 4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0078. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0078 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

### Storage and expiry date

Store unopened at 2 to 8° C. Do not freeze.

Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.

**Expiry date:** expiration date is on the box.

## 5. Sample preparation

1. Serum can be used as detected sample. Fresh collected samples should be fully centrifuged, then take clear liquid for test. Anticoagulant (such as EDTA, sodium citrate and heparin, etc.) in samples do not affect the results.
2. Samples with sodium azide cannot be detected. Because the sodium azide may inhibit the HRP activity. The suspended fibrous protein may cause a false positive if not fully precipitated. Avoid of samples with hyperlipidemia, hemolysis or jaundice. Obviously contaminated samples can't be detected.
3. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them below -20° C and avoid freeze-thaw cycles.

## 6. Assay procedure

Bring all reagents to room temperature for 30 min. Dilute the 20×Concentrated Wash Buffer for 20 times with distilled water.

## 1. **Add sample:**

- (1) Take out Micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 1 well for positive control (100  $\mu$ L control serum for each well). (Blank well is not necessary for dual-wavelength detection)
- (2) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (add 100  $\mu$ L of sample diluent and add 10  $\mu$ L of sample), mix fully.
- (3) Gently tap the plate to ensure thorough mixing.
2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
3. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
4. **HRP conjugate:** Add 100  $\mu$ L of HRP Conjugate working solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
6. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
7. **Add substrate:** Add 50  $\mu$ L of Substrate Reagent A and 50  $\mu$ L of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 10 min at 37° C in dark.
8. **Stop reaction:** Add 50  $\mu$ L of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. **OD Measurement:** Set the Micro-plate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

## 7. Reference value

### 1. **Result analysis**

- (1) Use each test result independently. Determine the result according to the Cut Off value.
- (2) Calculate the Cut Off:  $\text{Cut Off (C.O)} = 0.10 + \text{negative control (NC) average A value}$  (when NC average  $A_{450} < 0.05$ , calculate at 0.05; while NC average  $A_{450} \geq 0.05$ , calculate at the actual value).

### 2. **Quality control**

- (1) Blank well (just chromogenic agent and Stop Solution) absorbance  $\leq 0.08$ .
- (2) Positive control (PC)  $A_{450} > 0.50$ .
- (3) Negative control (NC)  $A_{450} < 0.08$ .

The experimental result is valid if quality control is valid.

### 3. Determination of results

- (1) Positive result: Sample absorbance  $\geq$  Cut Off.
- (2) Negative result: Sample absorbance  $<$  Cut Off.

## 8. Interpretation of results

1. Negative result indicates there is no AO antibody (IgG) detected in samples, while positive result means the opposite.
2. The positive result of AO antibody (IgG) is an important auxiliary index of diagnosis of infertility.

## 9. Limitations of test method

3. This test is only used as the qualitative detection of AO- IgG antibodies in serum of human.
4. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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