Human Ovarian antibody ELISA Kit

Enzyme Immunoassay for the quantification of antibodies to ovary antigens in serum.

Catalog number: ARG80366

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Antibodies directed against ovary antigens in serum can cause infertility in women. The application of the Human Ovarian antibody ELISA Kit is recommended for monitoring disorders of fertility and premature ovarian failure.

The Human Ovarian antibody ELISA Kit is a reliable and quantitative test for the determination of antibodies directed against human ovarial tissues. This test is intended for the use with serum.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A mix of ovary proteins has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated human antibody (Fc part of Immunoglobulins) is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard 1-4	4 vials (Ready-to-use) (6 U/ml, 25 U/ml, 50 U/ml, 100 U/ml)	4°C
Control (Accept range: 20.4 – 36.2 U/ml)	0.5 ml (Ready-to-use)	4°C
Dilution Buffer (Zero Standard)	50 ml	4°C
HRP-Antibody conjugate	8 ml (Ready-to-use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm (optional: read at 620 nm to 630 nm as reference wavelength)
- Incubator or oven for 37°C
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. Opened reagents must be stored at 2-8 °C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents and required number of strips to room temperature prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents must be mixed without foaming before use.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Once the test has been started, all steps should be completed without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at 4°C for up to 7 days or stored at ≤-20 °C for up to one year. Avoid repeated freeze-thaw cycles. Samples should be frozen only once at-20°C prior to assay.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Sera from patients with liver diseases should not be used

c) Samples containing sodium azide should not be used in the assay.

d) Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. E.g. Add 30 ml of 40 X Wash buffer into 1170 ml of distilled water to a final volume of 1200 ml. The diluted 1X Wash buffer is stable for 2 weeks at room temperature.
- Samples: Dilute samples at 1:100 dilution with Dilution Buffer before assay.

Example:

a) Dilution 1:10: 10 µL of sample + 90 µL of dilution buffer (mix thoroughly)

b) Dilution 1:100: 10 µL of 1:10 diluted a) + 90 µL of dilution buffer (mix thoroughly).

Note: The Quality Control is ready to use and must not be diluted!

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT; 20 °C to 26 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of standards, controls, samples and zero controls (dilution buffer) into appropriate wells.
3. Cover the plate with foil and incubate for 60 minutes at 37 °C.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher (400µl). Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 50 µl of HRP-antibody conjugate into each well. Cover the plate with foil and incubate for 60 minutes at 37 °C.
6. Wash as according to step 4, but wash for a total 5 washes.
7. Add 50 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark.
8. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450 nm (and reference filter 620 nm) immediately. It is recommended reading the absorbance within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

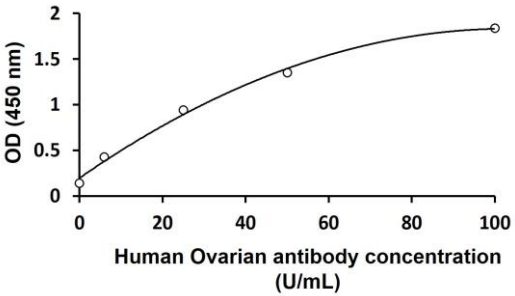
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The concentration of the samples can be read directly from this standard curve. The standards are already pre-diluted, therefore the 1:100 dilution of the samples must not be taken into account for the final calculation of sample concentrations.

7. Samples with concentrations higher than the highest standard can be further diluted or reported as > 100 U/mL. For the calculation of the concentrations this further dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



EXPECTED NORMAL VALUES

Normal Values: 0-10 U/ml

Elevated Values: >10 U/ml

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

QUALITY ASSURANCE

Standard Range

6-100 U/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.4% and inter-assay precision was 7.8%.