DRG® anti-Ovarian Ab ELISA (EIA-2937)

RUO in the USA

Revised 20 Mar. 2012 rm (Vers. 8.1)

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

Intended Use

The Anti-Ovarian Ab ELISA is a reliable test for measurement of antibodies directed against human ovarial tissues. This test is intended for the use with serum.

Principles of the Assay Method

The Anti-Ovarian Ab ELISA (Enzyme Linked ImmunoSorbent Assay) is a solid-phase sandwich enzyme immunoassay for the quantitative determination of anti-ovary antibodies in human serum.
A mix of ovary proteins is bound to the surface of the wells of the ELISA plate. The samples and standards are pipetted into the wells and then incubated. During this incubation anti-ovary antibodies in the sample bind to the ovary proteins and are thus immobilised on the plate. After several washing steps the enzyme conjugate, consisting of polyclonal antibodies directed against epitopes on the Fc part of human immunoglobulins and covalently coupled to horseradish peroxidase, is added. After removal of the unbound conjugate by washing the horseradish peroxidase oxidises the then added substrate TMB (3,3',5,5'-tetramethylbenzidine), yielding a colour reaction which is stopped by adding 0.25 M sulphuric acid (H_2SO_4). The extinction is measured at a wavelength of 450 nm with a microplate reader. The use of a reference measurement with a wavelength ≥ 550 nm is recommended.

Reagents

(sufficient for 96 determinations)

1.	**Microtiter strips coated** with ovary antigen	96 wells
2.	Ovary Antibody ELISA **Standard** set - per vial – Standard 1 (6 U/mL – colourless screw cap) – Standard 2 (25 U/mL – white screw cap) – Standard 3 (50 U/mL – yellow screw cap) – Standard 4 (100 U/mL – blue screw cap)	0.5 mL
3.	**Control** (green screw cap) equivalent to 15-50 U/mL	0.5 mL
4.	**Dilution Buffer** (also used as blank / zero standard / 0 U/mL)	50 mL
5.	**Wash Solution** (10x concentrated)	50 mL
6.	**Enzyme Conjugate** (ready for use)	5 mL

7.	**Substrate Solution** (solution of TMB, ready for use)	13 mL
8.	**Stop Solution** (0.25 mol/L H_2SO_4)	13 mL
9.	Holder for single strips	1 x

Materials Required but not Included

1. Microplate reader with 450 nm filter, optionally with a reference filter ≥ 550 nm.
2. Microliter pipettes with disposable tips: 5 µL, 10 µL, 50 µL, 100 µL, 500 µL and 1000 µL.
3. Tubes for the dilution of the samples
4. Distilled or deionized water
5. Absorbent paper.

Please use only calibrated pipettes and instruments.

Warnings and Precautions

1. Avoid contact with the stop solution; it may cause skin irritations and burns.
2. Do not pipette reagents by mouth.
3. Please regard all samples as potentially infectious and handle them with utmost care.
4. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where this exists.

Instructions for Reagent Preparation

1. The components of this kit are intended for use as an integral unit and should not be interchanged with the components of other kits.
2. All reagents and specimens must be brought to room temperature before use.
3. All reagents have to be mixed without foaming.
4. Once the test procedure has been started, all steps should be continued without interruption.
5. Pipette all reagents and samples onto the bottom of the wells. Mixing or shaking after pipetting is not required.
6. Use new disposable tips for each specimen.
7. Before starting the assay, all reagents to be used should be prepared and ready for immediate use, all needed strips should be secured in the holder etc. This will ensure equal time periods for each pipetting step without interruption.
8. For optimal results it is important to wash the wells thoroughly after incubation and to remove even the last water drops by hitting the plate on absorbent paper or cloth.

9. Since the kinetics of the enzymatic reaction depends on the surrounding temperature different extinctions correlating with the respective room temperature may be observed. The optimum laboratory room temperature is 20 °C – 22 °C (68 °F – 72 °F).
10. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

Storage Instructions and Shelf Life Information

1. Store the reagents at 2 °C – 8 °C (36 °F – 46 °F).
2. The reagents remain stable until the expiration date of the kit.
3. The diluted washing solution is stable for 4 weeks at refrigerator temperatures (2 °C – 8 °C / 36 °F – 46 °F).
4. Put caps back on the vials immediately after use.
5. Store the microtiter strips in a dry bag with desiccants. The remaining strips must be stored in the tightly resealed bag together with the desiccants. Under these storage conditions, they are stable at least for 4 weeks after opening of the sealed bag.

Sample Material

Human serum

Specimen Collection and Preparation

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature; avoid haemolysis. Avoid repeated freezing and thawing. Store tubes closed as they may be a danger of contamination or alteration of concentration.

1. Handle all samples with utmost care since they may be infectious.
2. There are no known interferences with extrinsic factors or other substances.
3. Samples may be stored at different temperatures for the following time-spans:
   - Environmental temperature up to 30 °C (86 °F): up to three days
   - Refrigerator temperature (2 °C – 8 °C / 36 °F – 46 °F): up to one week
   - Household freezer temperature (-10 °C to -20 °C / 14 °F to -4 °F): up to one year

ATTENTION! There are no test methods available which may guarantee that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including specimen samples, should be considered potentially infectious.

Assay Procedure

1. Warm all reagents to room temperature and mix thoroughly before use.
2. Preparation of the washing solution (10x): Dilute the concentrated washing solution (50 mL) by adding 450 mL distilled or deionized water. **Attention:** Do not use unpurified tap water!
3. Dilute sera 1:100 with dilution buffer (1:100 dilution: 5 µL of serum + 495 µL of dilution buffer).
4. Fix the required number of coated wells or strips in the strip holder.
5. Pipette 50 µL of standards into the respective wells.
6. Pipette 50 µL of diluted serum with new disposable tips into the respective wells.
7. Incubate for 60 min at 37 °C.
8. Briskly shake out the contents of the wells and then rinse the wells 3 times with 200 µL diluted washing solution each.
9. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
10. Dispense 50 µL of the enzyme conjugate into each well.
11. Incubate for 60 min at 37 °C.
12. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µL diluted washing solution each.
13. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
14. Dispense 50 µL of substrate solution immediately after the washing into each well.
15. Incubate for 30 min at room temperature.
16. Stop the enzymatic reaction by adding 50 µL of stop solution into each well in the same sequence and time interval as dispensing the substrate.
17. Measure the extinction of the samples at 450 nm. It is recommended to carry out the measurement of the extinction within 10 minutes after stopping the reaction.

As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions.
Since calibrators are assayed in each run, absorbance fluctuations do not affect the absolute results.

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Pipetting Scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	BLANK	P	3	P	11	P	19	P	27	P	35
B	S	1	P	4	P	12	P	20	P	28	P	36
C	S	2	P	5	P	13	P	21	P	29	P	37
D	S	3	P	6	P	14	P	22	P	30	P	38
E	S	4	P	7	P	15	P	23	P	31	P	39
F	P	C	P	8	P	16	P	24	P	32	P	40
G	P	1	P	9	P	17	P	25	P	33	P	41
H	P	2	P	10	P	18	P	26	P	34	P	42

In this pipetting scheme the recommended positions for the blank (please use the dilution buffer included in this kit), standards (S1 – S4), positive control (PC) and for the specimen samples (P1 – P42) are shown as double determinations.

Calculation of the Results

1. Calculate the average absorbance values for each set of reference standards, controls and specimen samples
2. The optical density of each standard value is plotted as y value (y-axis), the corresponding anti-ovary antibody value is drawn in as the x-value (x-axis). The resulting calibration curve is used to determine the values of the specimen samples. The OD values of the serum samples are correlated with the corresponding anti-ovary antibody concentration values by interpolation. A four parameter fit (sigmoid) should be used.
3. Using the mean absorbance value for each sample determine the corresponding concentration of anti-ovary antibody in U/mL from the standard curve.

Limitations of the Assay

- At temperatures higher than 30 °C (86 °F) the samples should be transported cooled or refrigerated. The time to stop the (enzymatic colour) reaction may have to be adjusted (shortened).
- Severely haemolytic or lipaemic sera or sera from specimens with liver diseases should not be used. Results may be adversely affected by certain pathologic conditions, such as poly- and monoclonal gammapathies, autoimmune diseases or by an altered immune status.