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User's Manual

Anti Ovarian Ab ELISA

Enzyme Immunoassay for the quantitative determination of antibodies directed against human ovarian tissues



DE2937



96 wells

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1. Intended Use

The Anti-Ovary Ab ELISA is a reliable and quantitative test for the determination of antibodies directed against human ovarian tissues. This test is intended for the use with serum.

2. Clinical Relevance

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

3. Fields of Application

The Anti-Ovary Ab ELISA can be applied in the clinical practice for the confirmation or the exclusion of antibodies against ovarian tissues as the cause of fertility problems.

4. Principles of the Assay Method

The Anti-Ovary Ab ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) 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 (H₂SO₄). 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.

5. Reagents

(sufficient for 96 determinations)

| | |
|--|----------|
| 1. Microtiter strips coated with ovary antigen | 96 wells |
| 2. Ovary Antibody ELISA standard set - per vial | 0.5 ml |
| – 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) | |
| 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. Washing 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 (0.5 N acidic solution) | 13 ml |
| 9. Holder for single strips | 1 x |

6. 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 μ l, 50 μ l, 100 μ l and 1000 μ l.
3. Tubes for the dilution of the samples.
4. Distilled or deionized water.
5. Absorbent paper.
6. Please use only calibrated pipettes and instruments.

7. Warnings and Precautions

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

8. 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.

9. 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.

10. Sample Material

Human serum

11. 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 – 8 °C / 36 °F – 46 °F): up to one week
 - Household freezer temperature (-10 °C – -20 °C / 14 °F – -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 patient samples, should be considered potentially infectious.

12. 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 150 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 150 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.

13. Pipetting Scheme for the Ovary Ab ELISA

| | 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 patient samples (P1 – P42) are shown as double determinations.

14. Calculation of the Results

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

15. 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 patients with liver diseases should not be used. Results may be adversely affected by certain pathologic conditions, such as poly- and monoclonal gammopathies, autoimmune diseases or by an altered immune status.

16. Expected Values

Normal values 0 - 10 U/ml
Elevated values above 10 U/ml

17. Assay Performance Characteristics










1. **Intraassay variation coefficient: 6.40% (5.50 – 7.80 %)**


For the determination of the intraassay variation coefficient 6 kits from 6 different batches (produced on different days) were used. One patient sample (optical density about 1.0) was applied 96 times per testing procedure.

2. **Interassay variation coefficient: 7.80% (5.50 – 9.60 %)**

For the determination of the interassay variation coefficient one strip each of 12 kits stemming from 6 different batches (produced on different days) were used. One patient sample (optical density about 1.0) was applied 72 times per testing procedure.

18. SYMBOLS USED WITH DEMEDITEC ASSAY'S

| Symbol | English | Deutsch | Français | Español | Italiano |
|---|------------------------------------|------------------------------|--|---------------------------------------|-------------------------------------|
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las instrucciones de uso | Consultare le istruzioni per l'uso |
|  | European Conformity | CE-Konformitätskennzeichnung | Conformité aux normes européennes | Conformidad europea | Conformità europea |
|  | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso Diagnóstico in vitro | Per uso Diagnostica in vitro |
|  | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
|  | Catalogue number | Katalog-Nr. | Numéro de catalogue | Número de catálogo | Numero di Catalogo |
|  | Lot. No. / Batch code | Chargen-Nr. | Numéro de lot | Número de lote | Numero di lotto |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze | Contenu suffisant pour "n" tests | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Storage Temperature | Lagerungstemperatur | Température de conservation | Temperatura de conservación | Temperatura di conservazione |
|  | Expiration Date | Mindesthaltbarkeitsdatum | Date limite d'utilisation | Fecha de caducidad | Data di scadenza |
|  | Legal Manufacturer | Hersteller | Fabricant | Fabricante | Fabbricante |
| Distributed by | Distributor | Vertreiber | Distributeur | Distribuidor | Distributore |
| Content | Content | Inhalt | Conditionnement | Contenido | Contenuto |
| Volume/No. | Volume / No. | Volumen/Anzahl | Volume/Quantité | Volumen/Número | Volume/Quantità |

| Symbol | Portugues | Dansk | Svenska | Ελληνικά |
|---|--------------------------------------|--|---|---------------------------------------|
|  | Consulte as instruções de utilização | Se brugsanvisning | Se bruksanvisningen | Εγχειρίδιο χρήστη |
|  | Conformidade com as normas europeias | Europaeisk overensstemmelse | Europeisk överensstämmelse | Ευρωπαϊκή Συμμόρφωση |
|  | Diagnóstico in vitro | In vitro diagnostik | Diagnostik in vitro | in vitro διαγνωστικό |
|  | | | | |
|  | Catálogo n.º | Katalognummer | Katalog nummer | Αριθμός καταλόγου |
|  | No do lote | Lot nummer | Batch-nummer | Αριθμός Παρτίδος |
|  | | Indeholder tilstrækkeligt til "n" test | Innehåller tillräckligt till "n" tester | Περιεχόμενο επαρκές για «n» εξετάσεις |
|  | Temperatura de conservação | Opbevarings-temperatur | Förvaringstemperatur | Θερμοκρασία αποθήκευσης |
|  | Prazo de validade | Udløbsdato | Bäst före datum | Ημερομηνία λήξης |
|  | Fabricante | Producent | Tillverkare | Κατασκευαστής |
| Distributed by | | | | |
| Content | Conteúdo | Indhold | Innehåll | Περιεχόμενο |
| Volume/No. | Volume/Número | Volumen/antal | Volym/antal | Όγκος/αριθ.. |