Product information

Information about other products is available at: www.ibl-america.com

Anti-Ovarian Ab ELISA

RUO

REF **IB79184**

Σ **96**

For research use only. Not for use in diagnostic procedures.

Manufactured for: Immuno-Biological Laboratories, Inc. (IBL-America)
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Please use only the valid version of the Instructions for Use provided with the kit.

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1 INTENDED USE

The **IBL-America Anti Ovarian Ab ELISA** is an enzyme immunoassay for the measurement of antibodies directed against oocytes in human serum.

For research use only. Not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The IBL-America Anti Ovarian Ab ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a mix of oocyte proteins. During incubation, anti-oocyte antibodies in the samples (standards, controls, samples) bind to the coated surface of the wells. A washing step removes unbound sample components.
Added enzyme conjugate binds to the immobilized antigen-antibody-complexes. The conjugate contains anti-human immunoglobulin antibodies, labelled with horseradish peroxidase (HRP).
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is proportional to the concentration of the analyte in the sample. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 WARNINGS AND PRECAUTIONS

- This kit is for research use only. For professional use only.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay. However, values for the samples will not be affected.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
- Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
- Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.

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- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-America.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with a mix of oocyte antigen.(incl. 1 sealing film)
2. **DIL Dilution Buffer / Zero Standard**, 1 vial, 50 mL, ready to use; Concentration: 0 U/mL Contains non-mercury preservative.
3. **CAL 1 - 4 Standard (Standard 1 - 4)**, 4 vials, 0.5 mL each, ready to use; Concentrations: 6 – 25 – 50 – 100 U/mL; Contain non-mercury preservative.
4. **CONTROL Quality Control**, 1 vials, 0.5 mL each, ready to use; For control values and ranges please refer to Certificate of Analysis (CoA). Contain non-mercury preservative.
5. **ENZ CONJ Enzyme Conjugate**, 1 vial, 8 mL, ready to use; Anti-human IgG antibody conjugated with horseradish peroxidase; Contains non-mercury preservative.
6. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).
7. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use; Contains 0.5 M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.
8. **WASH SOLN 40x Wash Solution**, 1 vial, 30 mL (40X concentrated); See “Reagent Preparation“.

Note: Additional Dilution Buffer for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Incubator for 37 °C
- Tubes for sample dilution
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 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.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Wash Solution

Add distilled water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any damage to the test kit or components, IBL-America must be informed in writing, at the latest one week after receiving the kit. Damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed of according to the official regulations.

5 SAMPLES COLLECTION AND PREPARATION

Serum can be used in this assay.
Note: Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use haemolytic, icteric, or lipaemic samples. For further information refer to chapter “Interfering Substances”.

5.1 Sample Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Individuals receiving anticoagulant therapy may require increased clotting time.

5.2 Sample Storage and Preparation

Samples should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying.
Samples stored for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay.
Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution

Prior to assaying, dilute each sample **1:100** with Dilution Buffer.
Example:
Dilution 1:100: 5 µL sample + 495 µL Dilution Buffer (mix thoroughly)
Note: The Quality Control is ready to use and must not be diluted!

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6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Optical density 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.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** each of **Zero Standard, Standard, Quality Control** and **diluted sample** with new disposable tips into appropriate wells.
3. Cover with foil and incubate for **60 minutes** at **37 °C**.
4. Rinse the wells **3 times** with **400 µL** diluted Wash Solution per well, if a plate washer is used.
   - OR -
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted Wash Solution per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense **50 µL Enzyme Conjugate** into each well.
6. Cover with foil and incubate for **60 minutes** at **37 °C**.
7. Rinse the wells **5 times** with **400 µL** diluted Wash Solution per well, if a plate washer is used.
   - OR -
   Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with **300 µL** diluted Wash Solution per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add **50 µL** of **Substrate Solution** to each well.
9. Incubate for **30 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **50 µL** of **Stop Solution** to each well.
11. Determine the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

6.3 Results

1. Determine the average optical density (OD) values for each set of standards, controls and samples.
2. Using graph paper, plot the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample to determine the corresponding concentration.
4. Automated method: The results in the Instructions for Use have been determined automatically using a 4-Parameter fit. (4-Parameter Rodbard or 4-Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.

The standards are already pre-diluted, therefore the 1:100 dilution of the samples must not be taken into account for the final determination of sample concentrations.

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7 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
The use of control samples is advised to assure the day to day validity of results.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

8 PERFORMANCE CHARACTERISTICS

8.1 Intra-assay

Mean CV: 6.40 % (range from 5.50 % – 7.80 %)
For the determination of the intra-assay precision, 6 kits from 6 different batches (produced on different days) were used. One sample (OD > 1.0) was applied 96 times per testing procedure.

8.2 Inter-assay

Mean CV: 7.80 % (range from 5.50 % – 9.60 %)
For the determination of the inter-assay precision one strip each of 12 kits stemming from 6 different batches (produced on different days) were used. One sample (OD > 1.0) was applied 72 times per testing procedure.

9 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Interfering Substances

Severely haemolytic or lipaemic sera or sera from subjects with altered liver values should not be used. Results may be adversely affected by factors such as poly- and monoclonal gammopathies, autoimmunity or immune status.

10 LEGAL ASPECTS

10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-America.

10.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore

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