Product information

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Anti-Spermatozoa Antibody (ASA) serum ELISA

RUO

REF **IB79154**

Σ **96**

For Research Use Only – Not for Use in Diagnostic Procedures

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1 INTENDED USE

The **IBL-America Anti-Spermatozoa Antibody (ASA) serum ELISA** is a manual enzyme immunoassay for the measurement of antibodies directed against human spermatozoa (ASA) in human serum.
This product is intended for research use only and not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The IBL-American anti-Spermatozoa Antibody (ASA) serum ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **sandwich principle.** The microtiter wells are coated with a mix of spermatozoa proteins. During incubation, anti-spermatozoa 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 laboratory professional use only.
- Before starting the assay, read the instructions for use completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- Do not mix or use components from kits with different lot numbers. It is advised not to interchange 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.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Do not reuse microtiter wells.
- Reagents of other manufacturers must not be used together with the reagents of this test kit.
- All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In that case, contact IBL-America.
- Microbial contamination of reagents or samples may give false results.
- 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.
- All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- 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 original vials as reagent contamination may occur.

General precautions

- Follow good laboratory practice and safety guidelines.
- 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 lab coats and disposable latex gloves when handling samples and reagents and where necessary safety glasses.

Biohazard information

- 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. However, no known test method can offer total assurance that no infectious agent is present.
- The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
- Bovine components originate from countries where BSE (Bovine spongiform encephalopathy) has not been reported.

- All materials and samples of human or animal origin must be handled as if capable of transmitting infectious diseases.
- Handling must be done in accordance with the procedures defined by appropriate national biohazard and safety guideline or regulation. Waste must be discarded according to local rules and regulations.

Information to chemical hazards and hazard classification

- Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
- Substrate Solution contains an ingredient in non-declarable concentrations which causes serious eye irritation. In case of possible contact with eyes, rinse immediately carefully and thoroughly with eye wash or water. After contact with skin, wash with plenty of water. Take-off contaminated clothing and wash it before reuse.
- Avoid contact with Stop Solution containing < 5 % H_2SO_4. It may cause skin irritation and burns.
- Chemicals and prepared or used reagents must be treated as hazardous waste according to the national safety guideline or regulation.
- This product does not contain substances which have carcinogenic, mutagenic or toxic for reproduction (CMR) properties.

All reagents of this test kit do NOT contain hazardous substances in concentrations to be declared, a classification and labelling is not required.
For detailed information, please refer to the Safety Data Sheet, which is available upon request directly from IBL-America.

4 MATERIALS

4.1 Materials provided with the kit

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with a mix of spermatozoa proteins
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: 31 – 62 – 125 – 250 U/mL; Contain non-mercury preservative.
4. **CONTROL Quality Control**, 1 vial, 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 < 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“.
9. Cover foil, Instruction for Use (IFU) and Certificate of Analysis (CoA)

4.2 Materials required but not provided

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

4.3 Storage and Stability of the Kit

Unopened kits and reagents as well as **opened reagents** must be stored at 2 °C to 8 °C.
The microtiter plate contains snap-off strips. Do not open the pouch of the wells until it reaches room temperature. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch including the desiccant and used in the plate frame provided. Once the foil bag has been opened, care must be taken to close it tightly again.
Once opened, reagent vials must be closed tightly again.

	Storage Temperature	**Stability**
Unopened kits and unopened reagents	2 °C to 8 °C	Until the expiration date printed on the label. Do not use reagents beyond this date!
Opened kit	2 °C to 8 °C	8 weeks

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.

Stability after dilution:	at 20 °C to 26 °C	1 week

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 must not be used for a test run. They have to be stored until a final solution has been found. After this, they must be disposed of according to the official regulations.

5 SAMPLE COLLECTION, STORAGE AND PREPARATION

The following sample material can be used in this test:

Human serum

Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use hemolytic, icteric, or lipemic samples. For further information refer to chapter "*Interfering Substances*".

*For the determination of anti-spermatozoa antibodies in seminal plasma please use our Anti-Spermatozoa Antibody (ASA) seminal ELISA (*REF *IB79155).*

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. Subjects receiving anticoagulants may require increased clotting time.
Whole blood should not be frozen before centrifugation.

5.2 Samples Storage

Samples must be stored tightly capped prior to performing the assay. If stored frozen, freeze only once. Thawed samples must be inverted several times prior to testing.

Stability	at 2 °C to 8 °C	4 days
	at -20 °C (in aliquots)	up to 12 months

5.3 Sample Preparation

Prior to assaying, dilute each sample **1:100** with *Dilution Buffer.*
Example:
Dilution 1:100: 5 µL sample + 495 µL *Dilution Buffer* (mix thoroughly)

Stability of diluted samples	at 2 °C to 8 °C	4 days
	at -20 °C (in aliquots)	8 months

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

6 ASSAY PROCEDURE

6.1 Procedural Notes

- All reagents and samples must be allowed to come to room temperature (20 °C to 26 °C) before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Use new disposal plastic pipette tips for each standard, control, or sample in order to avoid carry-over.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the test has been started, all steps must be completed without interruption and in the same sequence for each step.
- The enzymatic reaction is linearly proportional to time and temperature.
- Optical density is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in chapter “Test Procedure”.
- 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.
- During the incubation at 37 °C cover microtiter strips with foil to avoid evaporation.
- **Important note to wash procedure:**
  Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
- **Test performance using fully automated analysis devices:**
  Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.

6.2 Test Procedure

Each run must include a standard curve.
The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.
The given test procedure describes manual processing.

Important note: The accuracy of this assay is markedly influenced by the correct incubation temperature and incubation time.
Pipetting the samples should not exceed 15 minutes.

1. Secure the desired number of microtiter wells in the frame holder.
2. Pipette **50 µL** of each ***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. Wash the wells as follows:
   If the wash step is performed manually:
   Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with **300 µL** diluted *Wash Solution* per well.

   If an automated plate washer is used:
   Rinse the wells **5 times** with **400 µL** diluted *Wash Solution* per well.

   At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!
5. Add **50 µL** ***Enzyme Conjugate*** into each well.
6. Cover with foil and incubate for **30 minutes** at **37 °C**.
7. Wash as described in step 4.
8. Pipette **50 µL** of ***Substrate Solution*** to each well.
9. Incubate for **30 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
11. Measure the optical density (OD) of the solution in each well at **450 nm (measurement wavelength) and at 620 nm or 630 nm (reference wavelength for recommended background subtraction)** 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. The concentration of the samples can be read directly from the 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.
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and sample must be taken. If the two values deviate substantially from one another, IBL-America recommends retesting the samples.
3. Samples with concentrations exceeding the highest standard can be further diluted with *Dilution Buffer* and re-assayed as described in “Test Procedure”, or must be reported as > 250 U/mL. For the calculation of the concentrations, this dilution factor must be considered.
4. Automated method:
   The results in the instructions for use have been calculated automatically using a four-parameter logistic (4PL) curve fit. (4PL Rodbard or 4PL Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. Manual method:
   Using graph paper, construct a standard curve by plotting 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.
   Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.

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.
It is recommended to use control samples. The use of control samples is advised to assure the day-to-day validity of results..
The controls and the corresponding results of the Quality Control Laboratory are stated in the Certificate of Analyses (CoA) added to the kit. The values and ranges stated on the CoA always refer to the current kit lot and must be used for direct comparison of the results.
Apply appropriate statistical methods for analyzing control values and trends. If the results of the assay do not agree with 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 Specificity of Antibodies (Cross-Reactivity)

Until today, no substances are known to us, which have an influence to the measurement of Sperm Antibody in a sample.

8.2 Detection Capability

Limit of Blank (LoB)	5.440 U/mL
Limit of Detection (LoD)	8.553 U/mL
Limit of Quantification (LoQ)	12.386 U/mL
Measuring range	8.553 U/mL – 250 U/mL
Linear range	8.553 U/mL – 250 U/mL

8.3 Repeatability, Reproducibility

8.3.1 Repeatability

Determined with 4 samples covering the complete measuring range within 20 days in 2 independent runs per day. CV was calculated as mean CV of 40 runs. CV must be < 10 %.

Sample	n	Mean (U/mL)	CV (%)
1	40	39.68	3.9
2	40	77.99	3.0
3	40	106.16	4.2
4	40	155.95	3.9

8.3.2 Reproducibility (Between-run)

Determined with 4 samples covering the complete measuring range within 20 days in 2 independent runs per day and with 2 replicates per run (20 x 2 x 2). CV was calculated from 80 determinations. CV must be < 15 %.

Sample	n	Mean (U/mL)	CV (%)
1	80	39.68	11.2
2	80	77.99	8.7
3	80	106.16	11.4
4	80	151.86	13.1

8.3.3 Reproducibility (Between-lot)

Determined by 6 measurements of different samples with 3 different kit lots. CV must be < 15 %.

Sample	n	Mean (U/mL)	CV (%)
1	18	17.78	9.3
2	18	26.65	10.4
3	18	34.58	3.9
4	18	55.56	14.7

8.4 Recovery

Recovery was determined by adding increasing amounts of the analyte to different samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples. Acceptance range for recovery: 85 % to 115 %.

		Sample 1	Sample 2	Sample 3	Sample 4
Highest concentration added (U/mL)		24.76	24.76	24.76	24.76
Concentration (U/mL)		32.88	57.97	25.25	56.85
Average Recovery (%)		98.1	103.2	100.0	97.3
Range of Recovery (%)	**from**	93.5	91.6	89.2	94.1
	to	102.5	109.1	108.6	102.2

8.5 Linearity

Samples containing different amounts of analyte were serially diluted with *Dilution Buffer*. The percentage recovery was calculated by comparing the expected and measured values for the analyte. Acceptance range for recovery: 85 % to 115 %.

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 4
Highest Dilution		1:4	1:8	1:256	1:2	1:64
Concentration (U/mL)		79.03	162.20	217.33	216.49	444.36
Average Recovery (%)		101.2	99.9	93.4	102.4	93.4
Range of Recovery (%)	**from**	92.7	90.0	92.5	95.4	91.3
	to	109.6	112.3	94.1	108.4	98.3

9 LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use 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

9.1.1 Matrix Interference

No interference (bias < ± 20 %) was found for addition of interferent up to concentration stated in the table below.

Bilirubin, unconjugated	0.5 mg/mL
Hemoglobin	4 mg/mL
Triglyceride	7.5 mg/mL

9.1.2 Heterophilic Antibody Interference

This test is designed to minimize the influence of heterophilic antibodies by appropriate, high stringency sample diluent and 100-fold dilution of each sample. Nevertheless, complete suppression of their effects cannot be guaranteed (16).

9.1.3 Autoantibody Interference

Samples containing autoantibodies on average may show slightly increased mean values depending on the type. In consequence, more samples may be found within the grey-zone of the assay. This test is designed to minimize the influence of autoantibodies by appropriate, high stringency sample diluent and 100-fold dilution of each sample. Nevertheless, complete suppression of autoantibody effects cannot be guaranteed (16).

9.1.4 Interference by Substances

To date, we are not aware of any substance (drug) that could have an influence on the results of this assay.

9.2 High-Dose Hook Effect

"High-Dose Hook Effect" is not detected up to 5000 U/mL of ASA.

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. If there is any doubt or concern regarding a result, 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.

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-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	utilisation 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 catalogo
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	Température 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	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta