Instructions for Use

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Spermatozoa-Ab ELISA

Enzyme immunoassay for the quantitative determination of autoantibodies against surface antigens of spermatozoa in human serum.

REF **RE52029**

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EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the quantitative determination of autoantibodies against surface antigens of spermatozoa in human serum.

2. SUMMARY AND EXPLANATION

In Western Society, the incidence of infertile couples is estimated to be 10-15% of the population. The role of anti-sperm-antibodies in infertility remains controversial, due to the different methods of determination. Among the classical methods for detecting anti-sperm-antibodies, the sperm-agglutination test and the sperm-immobilisation test have been widely applied. These tests and other variants of agglutination tests are both time consuming and inconsistent in performance.
ELISA-tests for anti-sperm-antibodies have many advantages over conventional methods. The ELISA for spermatozoa-antibodies from IBL combines these advantages with high sensitivity and specificity. The test is easy to perform and enables the screening of large numbers of sera for immunological infertility.

3. TEST PRINCIPLE

The test is based on a non-competitive ELISA. Sperm-surface antigens are extracted from a pool by a method related to that of Alexander (1984) and are coated on the wells of microtiter strips. All samples are incubated in duplicate either on coated and also on uncoated wells (yellow plate).
The strips are incubated with diluted sera from patients, and after a washing step, are incubated again with peroxidase conjugated anti-human-immunoglobulin (IgA, IgG and IgM). Following a final wash step, enzyme substrate (TMB) is added and color developed is determined using an ELISA reader. The color intensity in the wells is proportional to the bound immunoglobulins. The non-specific binding is determined individually for each serum and subtracted from the total binding.
Results are read from a standard curve and are expressed as Units/mL (mU/ 100µL).

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to 6 w in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION AND STORAGE

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles.
Stability:	24 hours	6 months	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with sperm surface antigens.
1 x 100 µL	ENZCONJ CONC	**Enzyme Conjugate,** Concentrate (601x) Contains: anti-human IgG, IgA, IgM, conjugated to peroxidase.
3 x 1 mL	CAL LYO	**Standard,** lyophilized For exact concentrations see labels or QC certificate. For preparation of standard set.
3 x 1 mL	CONTROL LYO	**Control,** lyophilized Concentrations / acceptable ranges see QC certificate.
3 x 20 mL	DILBUF LYO	**Diluent Buffer,** lyophilized
2 x 50 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (20x) Contains: phosphate buffer, Tween, stabilizers.
2 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
1 x 12x8	MTP NSB	**Microtiter Plate (NSB)** Yellow Colored. Break apart strips. For determination of non-specific binding. Blocked with unspecific antigens. in foilbag with desiccant.
5 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 0-20 µL; 10-100 µL; 100-1000 µL
2. Disposable glass tubes (12 x 75 mm)
3. Incubator, 37 °C
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.

4. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

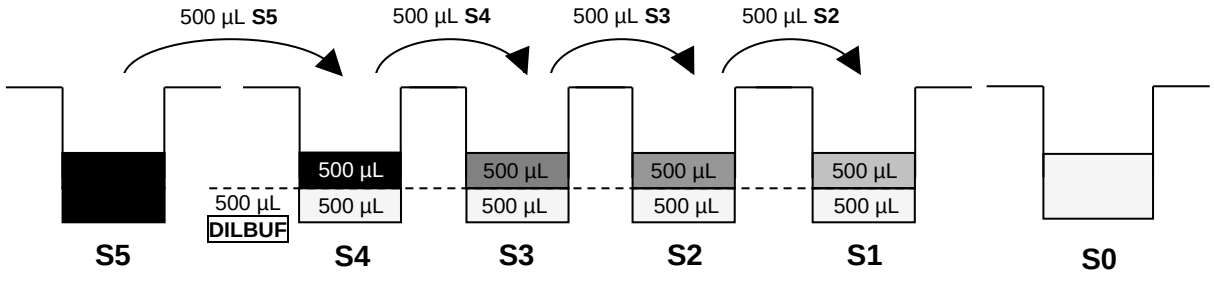
The volumes stated below are sufficient for one test procedure using 4 coated and 4 uncoated strips (32 determinations).

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
30 mL	**WASHBUF CONC**	ad 600 mL	bidist. water	1:20	Dissolve phosphate precipitates at 18-25°C.	2-8°C	4 weeks
15 µL	**ENZCONJ CONC**	with 9 mL	**DILBUF** (diluted)	1:601	Prepare freshly and use only once.	18-25°C	2 hours
	DILBUF LYO	with 20 mL	**WASHBUF** (diluted)			2-8°C ≤ -20°C	2 days 2 months
	CONTROL LYO	with 1 mL	**WASHBUF** (diluted)			2-8°C ≤ -20°C	2 weeks 4 weeks
	CAL LYO	with 1 mL	**WASHBUF** (diluted)			2-8°C ≤ -20°C	2 weeks 4 weeks

10.2. Preparation of Standards

Dissolve the content of one vial of lyophilized standard with 1 mL prepared **Wash Buffer** to obtain the stock standard solution (S5). Dilute S5 serially with Diluent Buffer according to the following scheme:

S5 = 500 µL of freshly dissolved standard = For exact concentration refer to certificate of analysis or vial label.
S4 = 500 µL S5 + 500 µL **DILBUF** (diluted) = Concentration of S5 / 2
S3 = 500 µL S4 + 500 µL **DILBUF** (diluted) = Concentration of S5 / 4
S2 = 500 µL S3 + 500 µL **DILBUF** (diluted) = Concentration of S5 / 8
S1 = 500 µL S2 + 500 µL **DILBUF** (diluted) = Concentration of S5 / 16
S0 = 500 µL **DILBUF** (diluted) = 0 mU/100 µL



10.3. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks	Storage	Stability
Serum	generally	**DILBUF** (diluted)	1:51	e.g. 10 µL + 500 µL Mix without foaming.	2-8°C	24 hours

Samples containing concentrations higher than the highest standard have to be diluted further.

11. TEST PROCEDURE

1.	Secure the coated and uncoated (NSB, yellow) microtiter strips alternately in the holder. For each Standard, Control and sample take 2 of the coated and 2 of the uncoated (yellow) wells.
2.	Pipette **100 µL** of each **Standard, Control and diluted sample** into the respective wells.
3.	Cover plate with adhesive foil. **Incubate 1 hour** at **37°C**.
4.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of freshly prepared **Enyzme Conjugate** into each well.
6.	Cover plate with new adhesive foil. **Incubate 1 hour** at **37°C**.
7.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
8.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
9.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
10.	**Incubate 10-15 minutes** at **18 - 25°C** (room temperature).
11.	Stop the substrate reaction by adding **50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
12.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **30 minutes** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

Correct the OD of the coated well for each standard, control and sample by the corresponding OD of the NSB well. Calculate the mean value of duplicates.

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

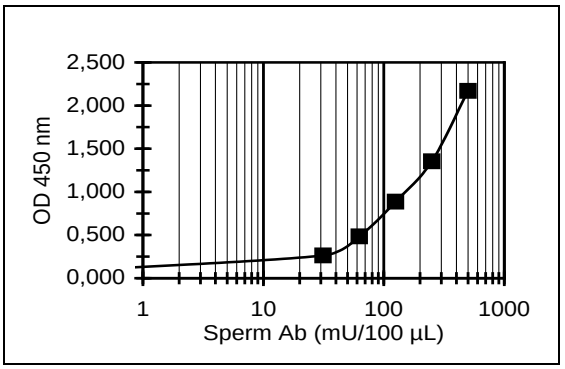
The concentration of the samples can be read directly from the standard curve.

Due to the dilution of samples the values obtained have to be multiplied by the factor 0.5 to receive the concentrations in U/mL in the undiluted sample.
In case of further diluted samples, the values have to be multiplied with the corresponding dilution factor.
Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and re-assayed.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Sperm-Ab. (mU/100 µL)	Mean OD_{coated}	Mean OD_{NSB}	OD_{coated} - OD_{NSB}
S0	0.0	0.066	0.009	0.057
S1	31.3	0.275	0.011	0.264
S2	62.5	0.493	0.009	0.484
S3	125	0.895	0.009	0.886
S4	250	1.371	0.018	1.353
S5	500	2.200	0.030	2.170



14. EXPECTED VALUES

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

Apparently healthy subjects show the following values:

< 150 mU/100 µL

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

For cross-reactivities, see PERFORMANCE.

16. PERFORMANCE

Analytical Specificity (Cross-reactivity)	No cross-reactivity was found with the typical substances tested.			
Analytical Sensitivity (Limit of Detection)	0.14 mU/100 µL	Mean signal (Zero-Standard) - 2SD		
Precision	Range (mU/100 µL)		CV (%)	
Intra-Assay	47.0 – 151.0		2.7 – 11.9	
Inter-Assay	42.0 – 130.0		7.9 – 15.4	
Linearity	Range (mU/100 µL)		Serial dilution up to	Range (%)
	31.4 – 376.9		1:8	95 - 110
Recovery	Mean (%)	Range (%)	% Recovery after spiking	
	90.7	77 - 101		
Method Comparison versus ELISA	IBL-Assay =4.0698 x ELIAS ELISA + 1.0441			r = 0.869; n = 38

17. PRODUCT LITERATURE REFERENCES

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4. F.M. Helmerhorst. M.J.J Finken. J.J Erwich: Antisperm antibodies: Detection assays for the antibody: What do they test?; Human reproduction. Vol.14. No.7. pp 1669 – 1673. 1999

5. Dona M. Lynch and Stephan E. Howe: Comparison of a Direct and Indirect ELISA for Quantitating Antisperm Antibody in Semen; Journal of Andrology July/August 1987; Vol.8. No.4; pp 215 – 220

6. Dana A. Ohl. M.D. and Alan C. Menge. Ph.D.: Assessment of sperm function and clinical aspects of impaired sperm function; Frontiers in Bioscience 1. e96-108. 1 September 1996; pp 96 - 108

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Store at: 2-8°C / Lagern bei: 2-8°C / Stocker à: 2-8°C / Almacene a: 2-8°C / Armazenar a: 2-8°C / Conservare a: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distribuidor: / Distributore: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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