BioVendor Group

ENG

Product Data Sheet:

HUMAN SPERM-ANTIBODY ELISA

Catalogue number:

RIS0017R

For research use only!

BioVendor R&D

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

CONTENTS

1. INTENDED USE 3
2. INTRODUCTION 3
3. TEST PRINCIPLE 4
4. REAGENT SUPPLIED 5
5. MATERIAL REQUIRED BUT NOT SUPPLIED 5
6. WARNING AND PRECAUTIONS 6
7. PREPARATION OF REAGENTS 7
8. STORAGE INSTRUCTIONS AND SHELF LIFE INFORMATION 7
9. SPECIMEN COLLECTION AND PREPARATION 7
10. ASSAY PROCEDURE 8
11. LIMITATIONS OF THE ASSAY 11
12. EXPECTED VALUES 11
13. PERFORMANCE CHARACTERISTICS 12
14. QUALITY CONTROL 14
15. REFERENCES 14

1. INTENDED USE

The RIS0017R Human Sperm-Antibody ELISA is an enzyme immunoassay for the measurement of antibodies directed against human spermatozoa in serum. It is intended for research use only.

The device is intended to be used as an aid for the diagnosis of immunologically caused disorders of fertility;

The device is not intended to be used for the detection of poly- and monoclonal gammopathies.

2. INTRODUCTION

Antibodies directed against spermatozoa antigens may cause infertility in women or men. The application of the Sperm Antibody ELISA is recommended for the diagnosis of immunologically caused disorders of fertility.

Unwanted childlessness is a growing problem with which up to 20% of all couples in the reproductive age are confronted temporarily or long-term. In 5-20 % of these cases the presence of anti-spermatozoa antibodies in the male or the female patient is detectable [1,2,15].

The definition of infertility according to the WHO (WHO Laboratory Manual for the Examination of Human Semen and Semen CervicalMucus Interaction, 1999) is the absence of a conception within 12 months of unprotected intercourse. The main cause of an immunological fertility disorder is the formation of antibodies directed against spermatozoa antigens.

Anti-spermatozoa antibodies (ASA) exert heterogeneous effects on the ability of spermatozoa to fertilize. The inhibiting effect of ASA on the motility of spermatozoa by binding to their surface and by agglutinating processes is well-known [3].

The penetration of the spermatozoa into the cervical mucus is impaired by the presence of ASA in the seminal plasma and/or in the cervical mucus [4]. ASA negatively influence the capacitation and the acrosome reaction of spermatozoa and thereby impede the interaction of the spermatozoa with the oocyte [5,6].

The interaction of the spermatozoon with the oocyte and the subsequent binding to and penetration of the zona pellucida may be inhibited by ASA. The following fusion of the oocyte and a spermatozoon may also be impaired by the presence of ASA [7,8].

The rate of pregnancies in couples with ASA on the part of the man or the woman was shown to be 38% lower compared to the control groups [9]. Furthermore an influence on the implantation and on the early embryological development could be confirmed. An association of ASA and miscarriages is discussed.

The frequency of ASA in infertile couples amounts to 20% [10,11].

ASA may occur dissolved in the ejaculate or bound to the surface of spermatozoa. ASA may be found in men and in women [12]. In women, ASA may be found in cervical mucus, oviduct liquid and follicular liquid. Men having more than 50% of their spermatozoa coated with anti-spermatozoa antibodies show a conspicuously reduced rate of fertility [13].

ASA have been shown to be associated with chronic prostatitis which has a negative effect on male reproductive function [14]

.

3. TEST PRINCIPLE

The BopVendor Sperm Antibody 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, patient specimen) 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.

4. REAGENT SUPPLIED

	Microtiters strips coated with sperm antigen 96 wells
CAL **N**	N=1 to 4 4 vials, 0.5 ml, ready to use Calibrator 1 (31 U/ml) Calibrator 2 (62 U/ml) Calibrator 3 (125 U/ml) Calibrator 4 (250 U/ml)
Ab **HRP**	Enzyme Conjugate 1 vial, 8 ml Anti-human IgG antibody conjugated with horseradish peroxidase ready to use
DIL **BUF**	1 vial, 50 ml/vial, ready to use Also used as blank / zero calibrator 0 U/ml
CHROM **TMB**	1 vial, 14 ml Ready to use Solution of TMB
STOP **SOLN**	1 vial, 14 ml, ready to use Contains 0.5M H2SO4
WASH **SOLN** **CONC**	1 vial, 30 ml (40X concentrated)
CONTROL **N**	N=1, 1 vial, 0.5 ml, ready to use
Holder for single strips 1 x	

5. MATERIAL REQUIRED BUT NOT SUPPLIED

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

6. WARNING 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 BioVendor. 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.

6.1 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.

6.2 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.

.

7. PREPARATION OF REAGENTS

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

8. STORAGE INSTRUCTIONS AND SHELF LIFE INFORMATION

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.

9. SPECIMEN COLLECTION 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 haemolytic, icteric, or lipaemic specimens. For further information refer to chapter “Interfering Substances”.

Specimen 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. Patients receiving anticoagulant therapy may require increased clotting time.

Specimen Storage

Samples must be stored tightly capped prior to performing the assay;

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

Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Prior to assaying, dilute each patient specimen 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)	7 days

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

10. ASSAY PROCEDURE

10.1 General Remarks

- 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 patient 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.

10.2 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 samples** 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 **30 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 **100 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*.

10.3 Calculation of 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 patient sample must be taken. If the two values deviate substantially from one another, BioVendor 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.

10.4 Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Density (450 nm)
(0 U/mL)	0.147
(31 U/mL)	0.515
(62 U/mL)	0.857
(125 U/mL)	1.423
(250 U/mL)	2.127

11. LIMITATIONS OF THE ASSAY

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.

Interfering Substances

Hemoglobin (up to 4 mg/mL), bilirubin (up to 0.5 mg/mL) and triglyceride (up to 7.5 mg/mL) have no influence on the assay results. 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.

High-Dose Hook Effect

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

12. EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently healthy subjects, using the BioVendor Sperm Antibody ELISA the following data were observed:

Normal values	0 – 60 U/mL
Borderline	55 U/mL – 65 U/mL
Elevated values	> 60 U/mL

In the case of a value in the range near the cut-off (55 U/mL to 65 U/mL) we recommend a follow-up determination using a new sample taken within the next two weeks.

The results alone should not be the only reason for any therapeutic consequences.

The results should be correlated to other clinical observations and diagnostic tests.

13. PERFORMANCE CHARACTERISTICS

Sensitivity

Limit of Blank (LoB)	0.490 U/mL
Limit of Detection (LoD)	3.367 U/mL
Limit of Quantification (LoQ)	9.632 U/mL
Measuring range	3.367 U/mL – 250 U/mL
Linear range	13.333 mL – 250 U/mL

Reproducibility

Within-run Precision

The within-run precision was determined with 4 patient samples covering the complete measuring range within 20 days in 2 independent runs per day. CV was calculated as mean CV of 40 runs.

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

Between-run Precision

The between-run precision was determined with 4 patient 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.

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

Between-lot Precision

The between-lot variation was determined by 6 measurements of different samples with 3 different kit lots.

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

Recovery

Recovery was determined by adding increasing amounts of the analyte to different patient samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (U/mL)		10.86	34.93	38.34	45.42
Average Recovery (%)		98.6	89.0	101.0	105.0
Range of Recovery (%)	**from**	90.3	86.2	92.0	99.0
	to	114.4	93.1	111.3	109.5

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.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (U/mL)		36.37	66.20	183.74	280.32
Average Recovery (%)		105.7	95.1	103.2	104.7
Range of Recovery (%)	**from**	103.9	92.3	99.7	98.6
	to	107.5	97.9	110.8	107.3

14. 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 according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

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 patient 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.

15. REFERENCES

1. Lahteenmaki A et a.: Hum Reprod (1995) 10, 2824-28
2. Nagy ZP et al. Hum Reprod (1995) 10, 1775-80.
3. Zouari R et al. Fertil Steril (1993) 59, 606-12
4. Eggert-Kruse W et al. Hum Reprod (1993) 8, 1025-31
5. Francavilla F et al. Front Biosci (1999) 4, 9-25
6. Bohring C et al. Hum Reprod (2001) 7,113-8
7. Mazumdar S et al. Fertil Steril (1998) 70, 799-810
8. Kutteh WH. Hum Reprod, (1999) 14, 2426-9
9. Vegetti Wet al. Hum Reprod (1998) 13, 1796-800
10. Lahteenmaki A et al. Hum Reprod (1995) 10, 2824-28
11. Nagy ZP et al. Hum Reprod (1995) 10, 1775-80
12. Clarke GN et al. Am J Reprod Immunol Microbiol (1985) 7, 143-7
13. Abshagen K et al. Fertil Steril (1998) 70, 355-6
14. Jiang Y et al. J Reprod Immunol (2016) 118:85-91
15. Lu SM et al. Asian J Androl (2019) 21(5):473-477

BioVendor R&D®

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com