

Instructions for use


Anti-Zona Pellucida Antibody Haemagglutination Test

Cat. No.: BS-20-30

Size: 40 Determinations
Storage: 2 °C – 8 °C (36 °F – 46 °F)

Agglutination test for the determination of the anti-zona pellucida antibody titer of
zona pellucida antibodies in human serum

- For *in-vitro* diagnostic use only -

.: EU Registration No.: DE/CA80/IVD1704

Certified Quality Management System according to

DIN EN ISO 9001:2000

Register No.: IC 373 038, certified by ifta-CERT

DIN EN ISO 13485

Register No.: CE 0483-0215; certified by mdc



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Also available from BIOSERV Diagnostics:

Gastroenterology	Order Code	Determinations per Kit
Elastase-1 ELISA (pancreatic insufficiency)	BS - 86 - 01	96
Pankrin ELISA (acute pancreatitis)	BS - 86 - 02	96
Infertility		
Sperm Antibody Latex Agglutination Test	BS - 10 - 10	50
Sperm Antibody ELISA	BS - 10 - 20	96
Sperm Antibody Haemagglutination Test	BS - 10 - 30	40
Sperm Antibody ELISA - Ig-Classifying	BS - 10 - 50	96
Zona Pellucida Antibody Latex Agglutination Test	BS - 20 - 10	50
Zona Pellucida Antibody ELISA	BS - 20 - 20	96
Zona Pellucida Antibody Haemagglutination Test	BS - 20 - 30	40
Zona Pellucida Antibody ELISA - Ig-Classifying	BS - 20 - 50	96
Ovary Antibody Latex Agglutination Test	BS - 40 - 10	50
Ovary Antibody ELISA	BS - 40 - 20	96
Ovary Antibody Haemagglutination Test	BS - 40 - 30	40
Ovary Antibody ELISA - Ig-Classifying	BS - 40 - 50	96
Monitoring of Risk Pregnancies		
IGF-BP1 ELISA (PP12)	BS - 30 - 10	96
Glycodelin ELISA (PP14)	BS - 30 - 20	96
Hormones, Tumor Markers		
FSH ELISA	BS - 85 - 21	96
HCG ELISA	BS - 85 - 22	96
LH ELISA	BS - 85 - 23	96
HPL ELISA	BS - 85 - 24	96
Prolactin ELISA	BS - 85 - 25	96
AFP ELISA	BS - 90 - 21	96

Intended Use

With the Anti-Zona Pellucida Antibody Haemagglutination Test from BIOSERV Diagnostics the titer of zona pellucida antibodies is detected in human serum.

Clinical Relevance

Antibodies directed against zona pellucida can inhibit the fertilization process and thereby cause infertility in women.

Fields of Application

The Anti-Zona Pellucida Antibody Haemagglutination Test from BIOSERV Diagnostics can be applied in the clinical practice for the confirmation or the exclusion of antibodies against zona pellucida as the cause of fertility problems.

Principles of the Assay Method

The test is based on the haemagglutination test principle using sensitised red blood cells (sSRBC) of sheep as target antigen. Antibodies in the samples agglutinate with sSRBC within 90 minutes.

Reagents

(sufficient for 40 determinations)

1. Antigen suspension (sSRBC)	0.45 ml
2. Positive control	0.30 ml
3. Negative control	0.20 ml
4. Dilution buffer (ready for use)	20 ml
5. Microplate	1 x

Materials Required but not Included

1. Tubes for the dilution of the samples.
2. Microliter pipettes with disposable tips: 10 µl, 20 µl and 50 µl.
3. Please use only calibrated pipettes.

Warnings and Precautions

4. This kit is intended for *in-vitro* use only.
5. Do not pipette reagents by mouth.
6. Please regard all samples as potentially infectious and handle them with utmost care.
7. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where this exists.

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. Use new disposable tips for each specimen.
6. Before starting the assay, all reagents to be used should be prepared and ready for immediate use. This will ensure equal time periods for each pipetting step without interruption.
7. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.
8. The optimum laboratory room temperature is 20 °C – 22 °C (68 °F – 72 °F).

Storage Instructions and Shelf Life Information

1. Store the reagents at 2 – 8 °C (36 °F – 46 °F).
2. The reagents remain stable until the expiration date of the kit.
3. Put caps back on the vials immediately after use.

Sample Material

Serum

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.

Assay Procedure

1. Inactivate the serum for 30 min at 56 °C.
2. Prepare a serial dilution of positive control using log 2 (1:4, 1:8, 1:16, 1:32).
3. Use the negative control undiluted.
4. Dilute serum initially 1:4 (100 µl of specimen + 300 µl of dilution buffer)
5. Dispense 50 µl of the diluted specimen, negative control and serial dilution of positive control (1:4, 1:8, 1:16, 1:32) into each well.
6. Add 10µl of antigen suspension (sSRBC) to each well.
7. Mix by tapping gently the plate with your fingertips.
8. Incubate microplate 90 min at room temperature without movement.
9. Inspect the wells visually for agglutination after 90 min. In case of a positive reaction the serum should be serially diluted in order to establish the titer of antibodies.

Evaluation of the Results

10. **Positive reaction:** After 90 min a positive result is indicated by a uniform, pale reddish appearance of the suspension with no visible formation of a circle or a pellet in the well.
11. **Negative reaction:** If there is a formation of a circle or a pellet in the centre of the well the result is negative. It is recommended to hold the microtiter plate in an angle of 45° to 80° to allow the sedimented sSRBC to move. Non-agglutinated erythrocytes will change their shape from “circle” to “triangle” within 2-3 minutes.

Inspect the wells visually for agglutination after 90 min. Compare reaction with your positive and negative controls.

The test should be repeated at least three times within 8 – 12 weeks in order to assess the clinical significance for immunological subfertility

Limitations of the Assay

- At temperatures higher than 30 °C (86 °F) the samples should be transported cooled or refrigerated.
- 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.