ZONA - PELLUCIDA - ANTIBODY - ELISA
IG TYPING KIT

Cat.-No. BS - 20 - 50

The **BIOSERV** Zona-Pellucida-ELISA-Ig-Typing-Kit is an indirect solid phase enzyme-linked immunoassay for the determination of immunoglobulin class specific antibodies against zona pellucida in serum.

Application

According to many studies antibodies directed against ZP antigens in serum can cause infertility of women or men.

Principle of test

The ZP-Antibody-Typing-ELISA is an indirect solid phase enzyme-linked immunoassay. The ELISA-plate is coated with a ZP specific antigen which recognises ZP antibodies. Zona pellucida antibodies from samples and standards bind to the antigen and are immobilised on the plate. An enzyme conjugate containing antiserum directed against different regions of human immunoglobulins of different classes (IgA, IgG, IgM) and POD binds to the antigen-antibody-complex during the incubation. Unbound conjugate is washed off with washing solution. In a further step the existing complex oxidises TMB being added by the substrate solution which is turning blue. The enzymatic colour reaction is stopped after a defined period of time. The concentration of oxidised TMB correlating proportionally to the concentration of ZP antibody is measured photometrically.

Reagents
(sufficient for 96 determinations)

1. Microtiter strips	
- coated with ZP antigen	96 wells
- uncoated	32 wells
2. Strong positive control, IgA, IgG, IgM	1 ml
3. Weak positive control, IgA, IgG, IgM	1 ml
4. Negative control, IgA, IgG, IgM	1 ml
5. Dilution buffer and zero standard	50 ml
6. Washing solution, concentrated 10x (mix before use with 450 ml distilled water)	50 ml
7. Enzyme conjugate (ready for use)	
- Anti-IgG	2.5 ml
- Anti-IgA	2.5 ml
- Anti-IgM	3.5 ml
8. Substrate solution (ready for use)	13 ml
9. Stopping solution (0.25 mol/l H_2SO_4)	12 ml
10. Holder for microtiter strips	1 x

Attention: Store the reagents at 2 - 8 °C.

Required materials but not included
- Microplate reader with 450 nm filter,
- Pipettes with disposable tips (5 µl, 10 µl , 50 µl, 100 µl, 500 µl , 1000 µl),
- Distilled or deionised water.

Specimen collection and preparation

Serum

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature; avoid haemolysis. Sera can be stored up to 2 days at 2 - 8 °C. If they can not assayed during this time they can be stored frozen at -20 °C.
Avoid repeated freezing and thawing. Store tubes closed as there may be a danger of contamination or alteration of concentration. Strong 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 gammapathies, autoimmune diseases or by an altered immune status.

ATTENTION! Test methods are not available which can offer complete assurance 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. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/ National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).

General remarks

1. Avoid contact with stop solution. It may cause skin irritation and burns.
2. Do not pipette reagents by mouth.
3. Replace caps on reagents immediately. Do not switch caps.
4. Store the microtiter strips in a dry bag with desiccants. The remaining strips must be stored in the tightly closed bag together with the desiccants. Under this storage conditions they are stable at least for 6 weeks.
5. The reagents remain stable until the expiry date of the kit.
6. The components of this kit are intended for use as an integral unit.
7. The components of different kits should not be mixed.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. All reagents and specimens must be allowed to get to room temperature before use. All reagents have to be mixed without foaming.
10. Once the test has been started, all steps should be continued without interruption.
11. Pipette all reagents and samples onto the bottom of the wells. Vortex-mixing or shaking of plates after pipetting is not required.
12. Use new disposable tips for each specimen.
13. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed strips secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

Test procedure

1. **Preparation of washing solution**: The concentrated washing solution (50 ml) must be diluted with 450 ml distilled water. The diluted washing solution is stable for 4 weeks at 4-8°C. **Attention:** The use of ultrapure distilled water is very important!
2. Dilute sera 1:100 with dilution buffer (dilution: 1:100: 5 µl of serum + 495 µl of dilution buffer).
3. Secure the desired number of coated strips in the holder.
4. Dispense 50 µl of controls into appropriate coated wells intended for control determination of IgA, IgM and IgG. Additionally for IgM control determination dispense 50µl of controls into uncoated wells.
5. Dispense 50 µl of diluted serum with new disposable tips into appropriate wells.
6. Incubate 60 min at 37°C.
7. Briskly shake out the incubation solution. Rinse wells 3 times with 150µl diluted washing solution.
8. Dispense 50 µl enzyme conjugate (Anti-IgA, Anti-IgG, Anti-IgM) into each well.
9. Incubate 60 min at 37°C.
10. Discard the incubation solution, rinse the wells 5 times with 150 µl diluted washing solution, and remove any residual (Tap the inverted plate on absorbent paper).

11. Add 50 µl of substrate solution immediately after the washing to each well.
12. Incubate the microtiter strips 30 min at room temperature.
13. Stop the enzymatic reaction by adding 50 µl of stop solution into each well with the same sequence adopted to dispense the substrate solution.
14. Determine the absorbency of each well at 450 nm with a microplate reader. It is recommended that the wells be read within 30 minutes following step 13.

Evaluation of the results

Any microplate reader capable of determining the absorbency at 450 nm may be used. The determination of the reaction of each patient sera is obtained as follows:

IgA / IgG:
The values of patients' sera are compared with those derived from the standards (negative, weak and strongly positive). The samples will be considered positive if the value is equal or higher to the value of the weak positive control. The value correlates with the intensity of the positive reaction i.e. the concentration of IgA /IgG in specimen.

IgM:
Subtract values of the colour reaction in uncoated wells from those of the control determination in coated wells. Compare the results with the values from patients' sera. The samples will be considered positive if the value is equal or higher to the value of the weak positive control. The value correlates with the intensity of the positive reaction i.e. the concentration of IgM in specimen.

Attention: Due to biologically caused titer changes only a repeated examination of patient, at least a thrice testing in intervals of 8 - 12 weeks, can contribute to detecting the immunological factors in sterility patients.

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