DRG® Zona Pellucida Ab Ig-Typing (EIA-3778)

CE IVD

Revised 20 Oct. 2005 **RUO in the USA**

Intended Use

The Anti-Zona Pellucida Antibody ELISA Ig-Classifying test from DRG is a reliable and quantitative test for the determination of immunoglobulin class specific antibodies directed against zona pellucida. This test is intended for the use with human serum. In the United States, this kit is intended for Research Use Only.

Clinical Relevance

Antibodies directed against zona pellucida antigens may cause infertility. Fertility disorders of unknown etiology to investigate the role of immunoglobulin classes (IgA, IgG, IgM) in women or men.

Fields of Application

The Anti-Zona Pellucida Antibody ELISA Ig-Classifying test from DRG can be applied in the clinical practice for the diagnosis of immunologically caused infertility in men and in women.

Principles of the Assay Method

The Anti-Zona Pellucida Antibody ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) Ig-Classifying test from DRG is a solid-phase sandwich enzyme-immunoassay for the quantitative determination of anti-zona pellucida antibodies in human serum.

The ELISA-plate is coated with a mix of ovary proteins which are recognized by anti-zona pellucida antibodies. The samples and controls are pipetted into the wells and then incubated. During this incubation anti-zona pellucida antibodies bind to the antigen and are thus immobilized 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. After removal of the unbound conjugate by washing the horseradish peroxidase oxidizes the then added substrate TMB (3,3',5,5'-tetramethylbenzidine) yielding a color reaction which is stopped with 0.25 M sulphuric acid (H_2SO_4). The extinction is measured at a wavelength of 450 nm with a microplate reader. The use of a reference measurement with a wavelength >550 nm is recommended.

Reagents

(sufficient for 96 determinations)

1.	Microtiter strips coated with zona pellucida antigen	96 wells
2.	Strong positive control, IgA, IgG, IgM	1.0 ml
3.	Weak positive control, IgA, IgG, IgM	1.0 ml
4.	Negative control, IgA, IgG, IgM	1.0 ml
5.	Dilution buffer (also used as blank / zero standard / 0 U/ml)	50 ml
6.	Washing solution (10x concentrated)	50 ml
7.	Enzyme conjugate (ready for use)	
	– Anti-IgG	2.5 ml
	– Anti-IgA	2.5 ml
	– Anti-IgM	2.5 ml
8.	Substrate solution (solution of TMB, ready for use)	13 ml
9.	Stop solution (0.25 mol/l H_2SO_4)	12 ml
10.	Holder for single strips	1 x

Materials Required but not Included

Microplate reader with 450 nm filter, optionally with a reference filter >550 nm.
Microtiter pipettes with disposable tips: 5 µl, 10 µl, 50 µl, 100 µl, 500 µl and 1000 µl.
Tubes for the dilution of the samples
Distilled or deionized water
Absorbent paper.
Please use only calibrated pipettes and instruments.

Warnings and Precautions

This kit is intended for *in vitro* use only. In the United States, this kit is intended for Research Use Only.
Avoid contact with the stop solution, it may cause skin irritations and burns.
Do not pipette reagents by mouth.
Please regard all samples as potentially infectious and handle them with utmost care.
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

The components of this kit are intended for use as an integral unit and should not be interchanged with the components of other kits.
All reagents and specimens must be brought to room temperature before use.
All reagents have to be mixed without foaming.
Once the test procedure has been started, all steps should be continued without interruption.
Pipette all reagents and samples onto the bottom of the wells. Mixing or shaking after pipetting is not required.
Use new disposable tips for each specimen.
Before starting the assay, all reagents to be used should be prepared and ready for immediate use, all needed strips should be secured in the holder etc. This will ensure equal time periods for each pipetting step without interruption.
For optimal results it is important to wash the wells thoroughly after incubation and to remove even the last water drops by hitting the plate on absorbent paper or cloth.
Since the kinetics of the enzymatic reaction depends on the surrounding temperature different extinctions correlating with the respective room temperature may be observed. The optimum laboratory room temperature is 20 °C – 22 °C (68 °F – 72 °F).
It is recommended to affect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

Storage Instructions and Shelf Life Information

Store the reagents at 2 °C – 8 °C (36 °F – 46 °F).
The reagents remain stable until the expiration date of the kit.
Put caps back on the vials immediately after use.
Store the microtiter strips in a dry bag with desiccants. The remaining strips must be stored in the tightly resealed bag together with the desiccants. Under these storage conditions, they are stable at least for 4 weeks after opening of the sealed bag.

Sample Material

Serum

Specimen Collection and Preparation

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature; avoid hemolysis. Avoid repeated freezing and thawing. Store tubes closed as they may be a danger of contamination or alteration of concentration.

Handle all samples with utmost care since they may be infectious.
There are no known interferences with extrinsic factors or other substances.
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

Warm all reagents to room temperature and mix thoroughly before use.
Preparation of the washing solution (10x): Dilute the concentrated washing solution (50 ml) by adding 450 ml distilled or deionized water. The diluted washing solution is stable for 4 weeks at refrigerator temperatures (4 °C – 8 °C / 39 °F – 46°F). **Attention:** Do not use unpurified tap water!
Dilute sera 1: 100 with dilution buffer (1:100 dilution: 5 µl of serum + 495 µl of dilution buffer).
Fix the required number of coated wells or strips in the strip holder.
Pipette 50 µl of controls into the respective wells intended for control determination of IgA, IgM and IgG.
Pipette 50 µl of diluted serum with new disposable tips into the respective wells.
Incubate for 60 min at 37 °C.
Briskly shake out the contents of the wells and then rinse the wells 3 times with 200 µl diluted washing solution.
Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
Dispense 50 µl of the enzyme conjugate (Anti-IgA, Anti-IgG, Anti-IgM) into each well.
Incubate for 60 min at 37 °C.
Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl diluted washing solution.
Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
Dispense 50 µl of substrate solution immediately after the washing to each well.
Incubate for 30 min at room temperature.
Stop the enzymatic reaction by adding 50 µl of stop solution into each well in the same sequence and time interval as dispensing the substrate.
Measure the extinction of the samples at 450 nm. It is recommended to carry out the measurement of the extinction within 10 minutes after stopping the reaction.

As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physicochemical conditions.

Since calibrators are assayed in each run, absorbance fluctuations do not affect the absolute results. In any case it is highly recommended to use an additional internal control if available.

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Pipetting Scheme for the Zona Pellucida Antibody ELISA Ig-Classifying Test from DRG

	IgG				IgM				IgA			
	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	BL	P5	P5	BL	BL	P5	P5	BL	BL	P5	P5
B	SPC	SPC	P6	P6	SPC	SPC	P6	P6	SPC	SPC	P6	P6
C	WPC	WPC	P7	P7	WPC	WPC	P7	P7	WPC	WPC	P7	P7
D	NC	NC	P8	P8	NC	NC	P8	P8	NC	NC	P8	P8
E	P1	P1	P9	P9	P1	P1	P9	P9	P1	P1	P9	P9
F	P2	P2	P10	P10	P2	P2	P10	P10	P2	P2	P10	P10
G	P3	P3	P11	P11	P3	P3	P11	P11	P3	P3	P11	P11
H	P4	P4	P12	P12	P4	P4	P12	P12	P4	P4	P12	P12

In this pipetting scheme the recommended positions for the blank (BL, please use the dilution buffer included in this kit), strong positive control (SPC), weak positive control (WPC), negative control (NC), and for the patient samples (P1 – P12) are shown as double determinations.

Calculation of the Results

Any microplate reader of determining the absorbency at 450nm may be used. The determination of the reaction of each patient sera is obtained as follows:
The values of patients' sera are compared with those derived from the controls (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/IgM in specimen.

It is important to note that a single test result does not necessarily have a clear diagnostic value, since considerable fluctuation of the antibody titer in a certain time interval can occur in some patients. Therefore it is recommended that the test should be repeated at least three times over a period of 8 – 12 weeks in order to judge the clinical relevance to immunological infertility.

Limitations of the Assay

At temperatures higher than 30 °C (86 °F) the samples should be transported cooled or refrigerated. The time to stop the (enzymatic color) reaction may have to be adjusted (shortened).

Severely hemolytic or lipemic 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.