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

Revised 1 Apr. 2010 rm (Vers. 3.0) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

Intended Use

The Anti-Zona Pellucida Antibody ELISA Ig-Classifying test 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.

Clinical Relevance

Antibodies directed against zona pellucida antigens may cause infertility. The Ig-classifying ELISA is used for the diagnosis of fertility disorders of unknown etiology in women.

Fields of Application

The Anti-Zona Pellucida Antibody ELISA Ig-Classifying test can be applied in the clinical practice for the diagnosis of immunologically caused infertility 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 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 sulfuric 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.	**Strong positive control**, IgA, IgG, IgM	1.0 ml
2.	**Weak positive control**, IgA, IgG, IgM	1.0 ml
3.	**Negative control**, IgA, IgG, IgM	1.0 ml
4.	**Dilution buffer** (also used as blank / zero standard / 0 U/ml)	50 ml
5.	**Washing solution** (10x concentrated)	50 ml

6.	**Enzyme conjugate** (ready for use) – Anti-IgG – Anti-IgA – Anti-IgM	 2.5 ml 2.5 ml 2.5 ml
7.	**Substrate solution** (solution of TMB, ready for use)	13 ml
8.	**Stop solution** (0.25 mol/l H_2SO_4)	13 ml
9.	**Microtiter strips coated** with zona pellucida antigen	96 wells
10.	**Holder** for single strips	1 x

Materials Required but not Included

1. Microplate reader with 450 nm filter, optionally with a reference filter ≥ 550 nm.
2. Microliter pipettes with disposable tips: 5 µl, 10 µl, 50 µl, 100 µl, 500 µl and 1000 µl.
3. Tubes for the dilution of the samples
4. Distilled or deionized water
5. Absorbent paper.

Please use only calibrated pipettes and instruments.

Warnings and Precautions

1. This kit is intended for *in vitro* use only.
2. Avoid contact with the stop solution; it may cause skin irritations and burns.
3. Do not pipette reagents by mouth.
4. Please regard all samples as potentially infectious and handle them with utmost care.
5. 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. Mixing or shaking after pipetting is not required.
6. Use new disposable tips for each specimen.
7. 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.

8. 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.
9. 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).
10. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

Storage Instructions and Shelf Life Information

1. Store the reagents at 2 °C – 8 °C (36 °F – 46 °F).
2. The reagents remain stable until the expiration date of the kit.
3. The diluted washing solution is stable for 4 weeks at refrigerator temperatures (4 °C – 8 °C / 39 °F – 46 °F).
4. Put caps back on the vials immediately after use.
5. 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.

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. Warm all reagents to room temperature and mix thoroughly before use.

2. Preparation of the washing solution (10x):
   Dilute the concentrated washing solution (50 ml) by adding 450 ml distilled or deionized water.
   Attention: Do not use unpurified tap water!
3. Dilute sera 1: 100 with dilution buffer (1:100 dilution: 5 µl of serum + 495 µl of dilution buffer).
4. Fix the required number of coated wells or strips in the strip holder.
5. Pipette 50 µl of controls into the respective wells intended for control determination of IgA, IgM and IgG.
6. Pipette 50 µl of diluted serum with new disposable tips into the respective wells.
7. Incubate for 60 min at 37 °C.
8. Briskly shake out the contents of the wells and then rinse the wells 3 times with 200 µl diluted washing solution.
9. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
10. Dispense 50 µl of the enzyme conjugate (Anti-IgA, Anti-IgG, Anti-IgM) into each well.
11. Incubate for 60 min at 37 °C.
12. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl diluted washing solution.
13. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
14. Dispense 50 µl of substrate solution immediately after the washing to each well.
15. Incubate for 30 min at room temperature.
16. 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.
17. 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 physico-chemical 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

	IgA				IgG				IgM			
	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 absorbance 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 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.

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Assay Performance Characteristics

1. Intra-assay variation coefficient: 6.80% (5.10 – 7.90 %)

For the determination of the intra-assay variation coefficient 6 kits from 6 different batches (produced on different days) were used. One patient sample (optical density about 1.0) was applied 96 times per testing procedure.

2. Inter-assay variation coefficient: 7.20% (5.50 – 9.00 %)

For the determination of the inter-assay variation coefficient one strip each of 12 kits stemming from 6 different batches (produced on different days) were used. One patient sample (optical density about 1.0) was applied 72 times per testing procedure.

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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Ογκος/αριθ..