DRG® Glycodelin ELISA (EIA-3779)

Revised 1 Dec. 2009 (Vers. 3.0) **RUO in the USA**

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

Intended Use

With the Glycodelin ELISA the quantitative measurement of Glycodelin (= placental protein 14) in human serum is possible.

Clinical Relevance

Human glycodelin, apart from other placenta proteins, is considered as biochemical marker for the progress of a pregnancy, especially for the activity of glandular epithelia at implantation and placentation. The level of glycodelin in serum provides information on the endometrium in relation to fertility The highest serum concentration can be found during the first trimester of the pregnancy. Pregnant women with irregular bleedings and reduced glycodelin level have a five times higher risk of habitual miscarriage compared to women with bleedings and normal glycodelin level. Women with the risk of habitual miscarriage during late luteal phase show lower glycodelin values than normal women. Serum levels in women with ectopic pregnancies are lower than those found in intrauterine pregnancies.
The concentration of glycodelin in serum is also an important parameter to monitor the menstruation cycle. As it can be used to distinguish between ovulatory and non-ovulatory cycles it helps in gaining important information for in vitro fertilisation.
During in vitro fertilisation glycodelin values are significantly higher in twin-pregnancies compared to single-pregnancies. Glycodelin in seminal fluid (up to 2 % of total protein) in addition with maternal endometrial glycodelin enables implantation and placentation by inhibiting an immune response against the allogeneic foetus. Values below 7 µg/ml can cause spontaneous abortion. Very high values of glycodelin in seminal fluid may also lead to male infertility.

Fields of Application

The Glycodelin ELISA can be applied in the clinical practice for
- pregnancy monitoring
- predicting an impending miscarriage
- fertility and infertility diagnostics
- in-vitro-fertilisation
- monitoring of the menstrual cycle

Principles of the Assay Method

The Glycodelin ELISA (Enzyme Linked ImmunoSorbent Assay) is a solid-phase sandwich enzyme-immunoassay for the quantitative determination of Glycodelin in human serum, menstruational fluid and amniotic fluid.
The ELISA-plate is coated with an antibody directed against glycodelin. Glycodelin from samples and standards bind to the antibodies and are immobilised on the plate. An enzyme conjugate containing another antibody directed against glycodelin and POD binds to the glycodelin-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 glycodelin is measured photometrically. 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 anti-Glycodelin antibodies	96 wells
2.	**Glycodelin standard set** per vial	0.5 ml
	– Standard 1 (3 ng Glycodelin/ml – colourless screw cap)	
	– Standard 2 (6 ng Glycodelin/ml – white screw cap)	
	– Standard 3 (25 ng Glycodelin/ml – yellow screw cap)	
	– Standard 4 (50 ng Glycodelin/ml – blue screw cap	
	– Standard 5 (100 ng Glycodelin/ml – brown screw cap	
3.	**Zero standard / dilution solution** (0 lU/ml)	20 ml
4.	**Enzyme conjugate** (monoclonal anti Glycodelin antibodies conjugated to horseradish peroxidase)	5 ml
5.	**Substrate solution** (solution of TMB, ready for use)	13 ml
6.	**Stop solution** (0.25 mol/l H_2SO_4)	13 ml
7.	**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: 50 µl and 100 µl.
3. Distilled or deionised water.
4. Absorbent paper.
5. 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 – 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.
4. 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 2 weeks after opening of the sealed bag.

Sample Material

Human 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

For the preparation of other sample materials please inquire drg@drg-diagnostics.de

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. Fix the required number of coated wells or strips in the strip holder.

3. Pipette 50 µl of each standard and of each patient sample into the respective wells.
   Samples of pregnant patients have to be diluted in a proportion of 1:20 in the dilution buffer.
4. Incubate for 90 minutes at room temperature.
5. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl distilled or deionised water.
6. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
7. Dispense 50 µl of the enzyme conjugate solution into each well.
8. Incubate for 30 minutes at room temperature.
9. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl distilled or deionised water.
10. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
11. Dispense 100 µl of substrate solution into each well.
12. Incubate for 15 min at room temperature (time from start of pipetting the substrate).
13. Stop the enzymatic reaction by adding 100 µl stop solution to each well, in the same sequence and time interval as dispensing the substrate.
14. 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.
If in a test run the absorbance of the 100 ng Glycodelin/ml-standard is lower than 1.0 the incubation time of the final enzymatic reaction may be extended.
If, on the other hand, the absorbance of the 100 ng Glycodelin/ml-standard is above the upper performance limit of the microplate spectrophotometer used the enzymatic reaction time may be reduced.
Since calibrators are assayed in each run, absorbance fluctuations do not affect the absolute results

Pipetting Scheme for the Glycodelin ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	0	P	3	P	11	P	19	P	27	P	35
B	S	1	P	4	P	12	P	20	P	28	P	36
C	S	2	P	5	P	13	P	21	P	29	P	37
D	S	3	P	6	P	14	P	22	P	30	P	38
E	S	4	P	7	P	15	P	23	P	31	P	39
F	S	5	P	8	P	16	P	24	P	32	P	40
G	P	1	P	9	P	17	P	25	P	33	P	41
H	P	2	P	10	P	18	P	26	P	34	P	42

In this pipetting scheme the recommended positions for the zero standard (S0), standards (S1 – S5), and for the patient samples (P1 – P42) are shown as double determinations.

Calculation of the Results

1. Calculate the average absorbance values for each set of reference standards, controls and patient samples.
2. The extinction of each standard value is plotted as y value (y-axis), the corresponding glycodelin value is drawn in as the x-value (x-axis). The resulting calibration curve is used to determine the values of the patient samples. The extinction values of the serum samples are correlated with the corresponding glycodelin concentration values by interpolation.
3. Using the mean absorbance value for each sample determine the corresponding concentration of glycodelin in ng/ml from the standard curve.
4. Any diluted samples must be further converted by the appropriate dilution factor.

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 colour) reaction may have to be adjusted (shortened).
- 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 gammapathies, autoimmune diseases or by an altered immune status.

Expected Values

Each laboratory should establish its own normal ranges based on patient population. The results provided below are based on randomly selected out-patient clinical laboratory samples:

Serum from normal donor	glycodelin concentration
male	< 10 ng/ml
female	< 15 ng/ml

In women the highest level can be detected in the secretory phase of menstrual cycle

Serum during pregnancy	glycodelin concentration
1. Trimester	12 - 100 ng/ml
2. Trimester	6 - 60 ng/ml
3. Trimester	6 - 30 ng/ml
non developing pregnancy	< norm
prognosis of early abort	< 16 ng/ml

In seminal plasma a concentration of < 9 µg/ml may be causative for male infertility.

Assay Performance Characteristics

Intraassay Variation Coefficients

In order to determine the intra- and the inter-assay variance single standards as well as samples from normal test persons with low and with medium levels of glycodelin were analyzed.

Sample	n	mean [ng/ml]	standard deviation [ng/ml]	coefficient of variation [%]
1	12	105.04	9.76	9.2
2	12	48.64	3.34	6.8
3	12	25.95	1.61	6.2
4	12	6.05	0.59	9.9
5	12	118.39	11.13	9.4

Interassay Variation Coefficients

Sample	n	mean [ng/ml]	standard deviation [ng/ml]	coefficient of variation [%]
1	18	99.60	3.90	3.92
2	18	50.17	2.07	4.17
3	18	24.90	0.84	3.38
4	18	6.05	0.11	1.90
5	6	52.07	4.97	9.56

Linearity

Spiked samples were used to determine the linearity of the assay. The sera from four different test persons were diluted with the zero standard in the ratios of 1:2, 1:4 and 1:8. Then the concentration of Glycodelin was determined and multiplied with the dilution factor.

Test person	glycodelin conc. in undiluted serum [ng/ml]	recovery		
		1/2 [%]	1/4 [%]	1/8 [%]
1	195	102	105	110
2	77	96	90	94
3	44	110	110	108
4	6	103	95	105

Recovery

A known concentration was added to the zero standard in a ratio of 1:2 and was then analysed.

Sample	Zero standard [ng/ml]	conc. [ng/ml]	expected value [ng/ml]	determined value [ng/ml]	recovery [%]
1	0	200	100.0	103.0	103.0
2	0	100	50.0	45.0	90.0
3	0	50	25.0	24.8	99.2
4	0	25	12.5	12.0	96.0

Detection Limit

The detection limit, defined as two standard deviations above the mean of the zero control (95% confidence interval), was found to be 6 ng/ml serum.

Cross Reactivities

The following hormones were tested for cross-reactivity of the assay:

		produced colour intensity equivalent to Glycodelin in serum [IU/ml]
Human chorionic gonadotropin (HCG)	2000 IU/l	0
Prolactin	200 µg/l	0
Human placenta lactogen (HPL)	20 µg/ml	0
Alpha Feta Protein (AFP)	300 mIU/ml	0

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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 tilstrækkeligt 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	Όγκος/αριθ..