DRG® IGF-BP I ELISA (IGF Binding Protein I) (EIA-3780)

REVISED 31 MAR. 2010 RM (VERS. 6.0)

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

1 INTENDED USE

With the IGF-BP1 ELISA the quantitative measurement of IGFBP1 (= placental protein 12) in human serum is possible.
For in-vitro diagnostic use only

2 CLINICAL RELEVANCE

Insulin-like growth factor binding protein 1 [also called: placenta protein 12 (PP12), BP-25, alpha1-pregnancy associated endometrial globulin (alpha1-PEG), somatomedin binding protein (3)] is a secretional product of the endometrium/decidua and is an acid-stable protein of 25 kDa. It can be detected in serum, amniotic fluid, follicle fluid and cerebrospinal fluid.
The amount of available IGF peptides in the maternal organism is minimized by protein-protein interaction with IGFBP1. As the Insulin-like action of the IGF peptides is inhibited elevated IGFBP1 concentrations can lead to retardation or intrauterine death of the foetus or miscarriage.
During the 3rd trimester of pregnancy values of IGFBP1 in maternal serum correlate negatively with the weight of the placenta and with birth weight.
On the other hand the amount of Insulin can influence the IGFBP1-concentration.; e.g. in case of Insulin deficiency (Diabetes mellitus), there will be a higher concentration of IGFBP1 in serum in comparison to lower levels in case of Insulin overproduction (Insulinom). By binding and neutralization of free IGF, an unlimited proliferation of trophoblasts into the deciduale endometrium is prevented.
Analysis of IGFBP1 in amniotic fluid is considered to be the best marker for the detection of fetal growth disorders.With the help of the determination of IGFBP1 retardations of the foetus can be detected much earlier than with ultrasonography. The highest concentration of IGFBP1 in amniotic fluid (50 µg/ml) is reached in the 2nd trimester.
In comparison to amniotic fluid the concentration of IGFBP1 in maternal or foetal serum is 100 to 500 times lower. In case of values above 100 µg/ml of maternal serum between 24th and 35th week of pregnancy, a disturbance of the foetal-placental unit is indicated. Moreover, the diagnosis of patho-histological alterations of the placenta is possible. Elevated concentration show up either as a relatively short peak or over a longer period of time. In both cases the gynaecologists has to consider the presence of a risk pregnancy. For monitoring of high risk pregnancies several examinations are recommended between the 24th and 35th week of pregnancy. Furthermore, IGFBP1 is important for the recognition of ruptures of the foetal membrane. In such a case, IGFBP1 values of vaginal secretion are approximately 1900 ng/ml (normal case 0.5 to 90 ng/ml).
From 10th to 20th week women with twin pregnancies after in vitro fertilization have significant higher IGFBP1 values than in case of single pregnancies. In accordance with the study multiple pregnancies do not reveal higher values than twin pregnancies, that means that the maximum value of secretorial capacity of the endometrium is obtained with twin pregnancy.
Due to the correlation of the IGF-BP 1 concentration in serum of children with their body size IGFBP1 has been proven to be a good indicator for growth disorders. In comparison to values of IGFBP1 concentration of normally grown children, children being constitutionally small have a mean value which is much higher.

3 FIELDS OF APPLICATION

The IGF-BP1 ELISA from DRG can be applied in the clinical practice for
- Assessment of insulin production beta cells

- Prediction of type II diabetes
- in vitro fertilization and pregnancy monitoring (twin pregnancies, growth disorders)
- Diagnosis of polycystic ovary syndrome
- Assessment of insulin production by beta-cells (insulin measurement is more complicated)
- Assessment of nutritional status

4 PRINCIPLES OF THE ASSAY METHOD

The IGF-BP1 ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) is a solid-phase sandwich enzyme-immunoassay for the quantitative determination of IGFBP1 in human serum.
The ELISA-plate is coated with a monoclonal antibody directed towards a unique antigenic site of an IGFBP1 molecule. IGFBP1 from samples and standards bind to the monoclonal antibody and are immobilized on the plate. An enzyme conjugate containing another monoclonal antibody directed towards a different region of IGFBP1 molecule and POD binds to the IGFBP1-antibody-complex during the incubation. Unbound conjugate is washed off with washing solution. After removal of the conjugate not bound by washing the horseradish peroxidase oxidizes the substrate TMB (3,3',5,5'-tetramethylbenzidine) yielding a color reaction which is stopped with 0.25 M sulphuric acid (H_2SO_4). The enzymatic color reaction is stopped after a defined period of time. The concentration of oxidized TMB correlating proportionally to the concentration of IGFBP1 in the serum 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.

5 REAGENTS

(sufficient for 96 determinations)

1.	**Microtiter strips** coated with monoclonal anti-IGFBP1 antibodies	96 wells
2.	**IGFBP1 standard set** - per vial Standard 1 (3 ng/ml – colorless screw cap) Standard 2 (12.5 ng/ml – white screw cap) Standard 3 (25 ng/ml – yellow screw cap) Standard 4 (50 ng/ml – blue screw cap)	0.5 ml
3.	**Dilution buffer / zero standard** (0 lU/ml – green screw cap)	5 ml
4.	**Enzyme conjugate**, ready for use (monoclonal anti IGFBP1 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

6 MATERIALS REQUIRED BUT NOT INCLUDED

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

7 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.

8 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 – 25 °C (68 °F – 77 °F).
10. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

9 STORAGE INSTRUCTIONS AND SHELF LIFE INFORMATION

- Store the reagents at 2 – 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.

10 SAMPLE MATERIAL

Human serum

10.1 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 °C – 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.

11 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.
4. Add 50 µl of enzyme conjugate to each well.
5. Incubate for 60 minutes at room temperature.
6. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl distilled or deionized water.
7. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
8. Pipette 100 µl of the substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Stop the enzymatic reaction by adding 100 µl stop solution to each well, in the same sequence and time interval as dispensing the substrate
11. 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 50 ng/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 50 ng/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.
In the case of samples exceeding 50 ng/ml a dilution with zero standard is possible.

Pipetting Scheme for the IGFBP1 ELISA

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

REVISED 31 MAR. 2010 RM (VERS. 6.0)

G	P	2	P	10	P	18	P	26	P	34	P	42
H	P	3	P	11	P	19	P	27	P	35	P	43

In this pipetting scheme the recommended positions for the zero standard (S0), standards (S1 – S4), and for the patient samples (P1 – P43) are shown as double determinations.

12 CALCULATION OF THE RESULTS

1. Calculate the average absorbance values for each set of reference standards and patient samples.
2. The optical density (OD, absorbance, extinction) of each standard value is plotted as y value (y-axis), the corresponding IGFBP1 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 OD values of the serum samples are correlated with the corresponding IGFBP1 concentration values by interpolation. A four parameter fit (sigmoid) should be used.
3. Using the mean absorbance value for each sample determine the corresponding concentration of IGBP1 in ng/ml from the standard curve.
4. Any diluted samples must be further converted by the appropriate dilution factor.

13 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 of the assay 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.

14 EXPECTED VALUES

It is recommended that each laboratory determines its own normal and abnormal range.

sample	**IGFBP1 concentration**
Normal donor	< 20 ng/ml
Normal pregnancy	
1. trimester	10 - 50 ng/ml
2. trimester	15 - 80 ng/ml
3. trimester	40 - 100 ng/ml

Due to greater individual differences it is recommend to monitor patients with more than 100 ng/ml before the conclusion can be drawn regarding pathological disturbances.

Pathology of the foetoplacental unit > 200 ng/ml
- intrauterine hypoxia
- intrauterine death
- hypotrophia of the foetus
- pre-eclampsia

Hyperplasia of the endometrium - sample menstrual fluid < 80 ng/ml
(normal range > 80 ng/ml)

Attention!
3 - 6 weeks after serum concentrations of IGFBP1 begin to increase a serious crisis of the pregnancy (growth retardation, abortion or intrauterine death) may occur. The IGFBP1 values should be used as an adjunct to other data available to the physician.

15 ASSAY PERFORMANCE CHARACTERISTICS

15.1 Cross reactivities

		produced color intensity equivalent to IGFBP1 in serum [ng/ml]
Human chorionic gonadotropin (HCG)	**2000 mIU/ml**	**0**
Prolactin	**200 ng/ml**	**0**
Human placenta lactogen (HPL)	**20 mg/l**	**0**
Alfa-Fetoprotein (AFP)	**300 IU/ml**	**0**

15.2 High-dose hook effect

No high-dose hook effect was observed in this test up to 1000 ng IGFBP-1 in serum samples.

REVISED 31 MAR. 2010 RM (VERS. 6.0)

BIBLIOGRAPHIE

[1] Bell S C, et al. Journal Endocrinology 118, (1988) 317-328
[2] Waites G T, et al. Journal Clin. Endocrinology Metab. 67, (1988) 1100-1104
[3] Koistinen R, et al. Immunology 69, (1986) 1375-1378
[4] Seppälä M, et al. Journal Clin. Endocrinology Metab. 58, (1984) 505-510
[5] Fowelin J, et al. Acta Diabetologica 31, 4 (Dec 1994) 183-186
[6] Pedersen J F, et al. Brit. Jour. of Obst. and Gyn. 102, 11 (Nov 1995) 927-928
[7] Ibanez L, et al. Journal Clin. Endocrinology Metab. 82, 7 (Jul 1997) 2283-2288
[8] Rajaratnam V S, et al. Journal Endocrinology 152, 1 (Jan 1997) R 1-R 6
[9] Verhaeghe J, et al. Amer. Jour. of Obst. and Gyn. 175, 5 (Nov 1996) 1180-1188
[10] Miell J P, et al. Journal Clin. Endocrinology Metab. 82, 1 (Jan 1997) 287-292
[11] Giudice L C, et al. Amer. Jour. of Obst. and Gyn. 176, 4 (Apr 1997) 751-757
[12] Wathen N C, et al. Journal Endocrinology 137, 2 (May 1993) R 1-R 4
[13] Hakalaalapietila TH, et al. Amer. Jour. of Obst. and Gyn. 169, 1 (Jul 1993) 35-39
[14] Meisel M, et al. Zentralblatt für Gynäkologie 115 (1993) 383-387
[15] Römer Th, et al. Immun. Infekt. 22, 2 (1988) 55-57
[16] Baldwin S, et al. Journal Endocrinology 136, 2 (Feb 1993) 319- 325
[17] Hills F A, et al. Journal Endocrinology 148, 2 (Feb 1996) 303-309
[18] Abbas A, et al. Human Reproduktion 10, 1 (Jan 1995) 207-210
[19] Vandessel HJHMT, et al. Journal Clin. Endocrinology Metab. 81, 3 (Mar 1996) 1224-1231
[20] Homburg R, et al. Human Reproduktion 7, 10 (Nov 1992) 1379-1383
[21] Buyalos RP, et al. Amer. Jour. of Obst. and Gyn. 172, 3 (Mar 1995) 932-939
[22] Rutanen E M, et al. Clinica Chimica Acta 214, 1 (Jan 1993) 73-81
[23] Lockwood C J, et al. Amer. Jour. of Obst. and Gyn. 171, 1 (Jul 1994) 146-150
[24] Harding S, et al. Brit. Jour. of Obst. and Gyn. 102, 11 (Nov 1995) 891-893
[25] Verhaeghe J, et al. Amer. Jour. of Obst. and Gyn. 169, 1 (Jul 1993) 89-97
[26] Bang P, et al. Pediatric Research 36, 4 (Oct 1994) 528-536
[27] Hill F A, et al. Early Human Development 44, 1 (Jan 1996) 71-76
[28] Osorio M, et al. Early Human Development 46, 1-2 (Sep 1996) 15-26
[29] Bernardini S, et al. Acta Endocrinologica 127, 4 (Oct 1992) 313-318
[30] Lindgren B F, et al. Growth Regulation 6, 3 (Sep 1996) 158-164

REVISED 31 MAR. 2010 RM (VERS. 6.0)

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..