



IGFBP-1 Elisa

KAPME01

LOT : 110624/1



IGFBP-1-ELISA

Enzyme Immunoassay for the Quantitative Determination of
Human Insulin-like Growth Factor Binding Protein-1

KAPME01

IN VITRO DIAGNOSTIC USE

en

DIAsource ImmunoAssays S.A. - Rue du Bosquet 2, B-1348 Louvain-la-Neuve, Belgium - Tel: +32 10 84 99 11 - Fax : +32 10 84 99 90

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TECHNICAL FEATURES+APPLICATIONS

- ◆ **Quantitative determination of IGFBP-1** in serum and in other body fluids, like e.g. amniotic fluid, milk, urine or saliva etc., and in cell culture media.
- ◆ extremely high **analytical sensitivity of 0.02 ng/ml**
- ◆ Inter-Assay variation of 7,4% and Intra-Assay variation of 6,8%
- ◆ results available in **only 1.75 h incubation time**

INTRODUCTION

The Insulin-like Growth Factors I and – II are free in body fluids and tissues but are bound to specific binding proteins. Until today seven different binding proteins (IGFBP-1 to –7) can be differentiated additionally several IGFBP-related proteins have also been detected. Bioavailability of IGF is regulated by these IGFBPs or better their proteolytic cleavage which reduces affinity to IGF. But the IGFBPs as well as their proteolytic fragments can also exert IGF-independent effects, like influencing cell migration or proliferation.

IGFBP-1 (Placental Protein 12) consists of 234 aminoacids and has a molecular weight of approximately 25kDa. The coding DNA region is located on chromosome 7 [1, 2]. IGFBP-1 is mainly synthesized by foetal and adult liver tissue and decidual endometrium. Intensity of Expression varies enduring menstruation with a maximal expression in the late secretory phase [3, 4]. Further IGFBP-1 expression seems to be regulated by Insulin concentration, with Insulin inhibiting the expression. Insulin regulation results in diurnal fluctuations of up to factor 10 [5]. IGFBP-1 is posttranslational modified by phosphorylation of serine residues 101, 119 and 169. Phosphorylation has physiological relevance as it increases affinity of IGFBP-1 to IGF. In adult humans phosphorylated IGFBP-1 of the liver is the predominant form in circulation. IGFBP-1 produced by endometrial tissue is significantly less phosphorylated than the liver originated form [6].

In pregnancy IGFBP-1 maternal serum concentration increases significantly with maximal values in the second trimester or 22-23 week of gestation (75.8 ng/ml) [5] and decreases slowly until term. IGFBP-1 concentration are not only increased in maternal but also in foetal serum and with extremely high concentrations in amnion fluid. Here concentration can reach more than the 1000-fold of serum values [7]. Long-term changes of serum IGFBP-1 concentration can also be found in amnion fluid: IGFBP-1 level of the child decreases after birth until it reaches the low steady-state level of puberty and adulthood [8, 9].

Short term IGFBP-1 serum concentration is strongly influenced by nutrition level and therewith by insulin. Decreasing IGFBP-1 levels can be found enduring fasting or in diabetes; IGFBP-1 levels increase in case of intensive exercises [10-12].

Relevance of serum and amnion IGFBP-1 in diagnostics has been investigated in several areas. A diagnostic value was assigned for trisomy 18, intrauterine growth retardation, endometrial tumors and pre-eclampsia [14].

Thoroughly investigated was the diagnostic value in insulin resistance and pre-term rupture of the membrane and specially in the second field a significant diagnostic value could be demonstrated.

- **Energy metabolism**

Based on the influence of Insulin on IGFBP-1 serum concentrations IGFBP-1 is said to be a possible marker for insulin resistance. Because measurement of IGFBP-1 is much easier facilitated than Glucose – uptake rate this would simplify diagnosis of insulin resistance.

In a small study Maddux et al were able to demonstrate with 23 non-diabetic patients, that IGFBP-1 serum concentration correlated very well with Glucose-uptake rate, even better than the HOMA index does [13].

- **Pregnancy**

In pregnancy a significant difference in IGFBP-1 serum concentration of healthy pregnant and diabetic and pre-eclamptic women was found (102,8 vs. 203,71 or 281,09 ng/ml respectively) [15].

Also the evaluation of IGFBP-1 as marker for membrane rupture showed a high specificity (97%) and sensitivity (75%) of IGFBP-1 in vaginal/cervical secrets. In case of intact membrane IGFBP-1 concentration was < 90ng/ml in the secretion. Enduring 8 hours after spontaneous or induced membrane rupture IGFBP-1 values increased significantly with a median concentration of 1900 ng/ml. In this study IGFBP-1 concentrations von >100ng/ml were set as threshold for detection of amnion fluid and therewith diagnosis of membrane rupture [16]. A positive predictive value of 97% clearly shows that IGFBP-1 is a suitable marker for premature membrane rupture [17].

INTENDED USE

This enzyme immunoassay kit is suited for measuring IGFBP-1 in human serum or Heparin-plasma or in other body fluids, for example amnion fluid, mother milk, urine or saliva, as for diagnostic and scientific purposes. It is also suited to quantitate IGFBP-1 in cell culture media.

PERFORMANCE CHARACTERISTICS and Validation

The DIAsource **IGFBP-1 Elisa KAPME01** is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGFBP-1 in the sample binds to the immobilised first antibody on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-IGFBP-1-Antibody binds in turn to the immobilised IGFBP-1. Finally, the bound peroxidase catalyses the substrate reaction resulting in a colored product. Therefore colour intensity is highly specific and quantitatively depending on the IGFBP-1-level of the samples.

The calibrators of the ELISA KAPME01 are **native human IGFBP-1** in concentrations of **0, 0.1, 0.5, 1, 2, 4 and 8 ng/ml**.

The **analytical sensitivity** of the ELISA KAPME01 yields **0.02 ng/ml** (equal to **2 pg per well**; 2 SD of zero calibrator in 22 fold determination).

The determination of IGFBP-1 with DIAsource ELISA KAPME01 is over a very wide range authentic in dilution. The **linearity of serum dilutions** is over a wide range **excellent** (table 1).

Table 1: Linearity of Dilution (typical results of 2 different sera)

Dilution:	sample 1 (re-calculated, ng/ml)	Dilution:	sample 2 (re-calculated, ng/ml)
1:2.5	14.38	1:2.5	16.81
1:5	14.22	1:5	15.51
1:10	13.42	1:10	16.22
1:20	13.81	1:20	14.45
1:40	13.11	1:40	15.12
1:80	12.52	1:80	13.43
1:160	14.65	1:160	15.95
AV / 1SD / CV%	13.73 / 0.76 / 5.53	AV / 1SD / CV%	15.36 / 1.14 / 7.44

AV = average value, SD = calibrator deviation, CV = coefficient of variation

The **recovery** of native IGFBP-1 in different sample matrices is listed in table 4.

The measured **cross reactivity** for recombinant IGFBP-2 as well as IGFBP-3 was found to be negligible, measured in 500 ng/ml each, **less than 0.0015%** were quantitated.

The **Inter-** and **Intra-Assay** coefficients of variation were found less than **7.4% and 6.8%**. Exemplary determinations are shown in table 2 and table 3.

Table 2: Inter-Assay-Variation

	Average Value (ng/ml)	Calibrator Deviation (ng/ml)	Coefficient of Variation (%)
Sample 1	2.31	0.12	5.23
Sample 2	18.41	1.36	7.36
Sample 3	32.79	2.22	6.75

Table 3: Intra-Assay-Variation

	Average Value (ng/ml)	Calibrator Deviation (ng/ml)	Coefficient of Variation (%)
Sample 1	1.45	0.08	5.87
Sample 2	20.64	1.29	6.23
Sample 3	162.99	11.09	6.81

The comparison of IGFBP-1 determinations of 35 sera from healthy adults with the DIAsource ELISA KAPME01 and another commercially available ELISA yields a **very high Correlation** of the measured values: $y = 1,15x + 0,12$; $r^2 = 0,94$

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum samples, EDTA- and Heparin-Plasma samples are suitable. A special external sample preparation prior to assay is not required. Results in Citrate-Plasma are about 15% reduced. Slight hemolysis of the samples doesn't disturb the determination.

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen -20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although IGFBP-1 levels were found to be unaffected by few cycles(3x) in our experiments.

In most determinations (e.g. Serum- or Plasma samples and no extreme values expected, see table 4 for further details) the dilution of **1:16 with Dilution Buffer is suitable**, the respective covered range would be 0 to 128 ng/ml.

Suggestion for dilution protocol:

Pipette 300 µl **Dilution Buffer** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **20 µl Serum- or Plasma** (dilution 1:16) and mix each tube **immediately**. After mixing use **50 µl** of this solution within 1 hour **per determination** in the assay.


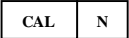


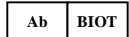
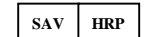
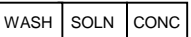
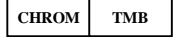
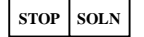
Where required, depending on the expected IGFBP-1-values, the dilution with Dilution Buffer can be higher or lower (at least however 1:2.5). The IGFBP-1 concentrations maybe completely different in body fluids of human origin other than serum or in cell culture supernatants. Examples as well as dilution recommendations are given in table 4.

Table 4: Sample matrices, recovery and dilution recommendation

Samples	Concentration IGFBP-1 (ng/ml)	Recovery of added IGFBP-1	Recommended Dilution as Sample in KAPME01
Amniotic Fluid	8,140.0 16,450.0	n.d.	Individually different. At least 1 : 5000 up to 25000
Mother Milk	5.12 20.2	91% (at 1:10 dil.) n.d.	1:10
Urine	0.07	89,8% (at 1:2.5 dil.)	1:2.5
Saliva	< 0.02 ng/ml	62,5% (at 1:2.5 dil.)	at least 1:2.5
Bronchial Lavage	< 0.02 ng/ml	100% (at 1:2.5 dil.)	1:2.5
Sputum	< 0.02 ng/ml	100% (at 1:20 dil.)	1:20
Serumpool	0.57	105.1% (at 1:16 dil.)	1:16 (general recommendation)
Pregnancy sera	n.d.	n.d.	1:25
Cell Culture Media	individually different	94,5% (at 1:5 dil.)	individually different at least 1:5

n.d.= not determined

REAGENTS PROVIDED

- 1)  **Microtiter plate**, ready for use: **Microtiter plate** with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-IGFBP-1 antibody and packed in a laminate bag.
- 2)  **Calibrators 0-6**, lyophilized: contain native human IGFBP-1. Calibrators values are between 0 – 8 ng/ml (0, 0.1, 0.5, 1, 2, 4 and 8 ng/ml) IGFBP-1, Calibrators are reconstituted with 500 µl Dilution Buffer each. Use 50 µl per well in the assay.
- 3)  **Dilution Buffer**, 125 ml, ready for use, please use for dilution of samples, control and calibrators.
- 4)  **Control 1 and 2**, 250 µl, lyophilised, contain human Serum and should be reconstituted in 250 µl Dilution Buffer each. The IGFBP-1 target values and the respective ranges are given on the vial label. The dilutions should be according to the dilution of the respected samples. Use 50 µl per well in the assay.
- 5)  **Biotin Conjugate**, 6 ml, contains biotinylated anti-human IGFBP-1 Antibody. Use 50 µl per well in the assay.
- 6)  **Streptavidin HRP**, 12 ml, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin. Ready for use. Use 100 µl per well in the assay.
- 7)  **Washing Buffer**, 50 ml, 20 X concentrated solution. Dilute 1:20 with Aqua dest. The 1:20 diluted Washing Buffer is only limited stable. Please dilute only according to daily requirements.
- 8)  **TMB-Substrate Solution** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H₂O₂₈ Tetramethylbenzidine. Use 100 µl per well in the assay.
- 9)  **Stopping Solution**, 12 ml, ready for use, 0.2 M sulphuric acid. Use 100 µl per well in the assay.
- 10) **Sealing tape** for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips
 Distilled or Deionized water for dilution of the Washing Buffer
 Vortex-mixer
 Device to aspirate the calibrators and the samples from the wells (recommended because of the potential danger of infection by human samples)
 Timer (120 min. range)
 Reservoirs (disposable)
 Plate washer and plate shaker (recommended)
 Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)
 Foil welding device for laminate bags (recommended)

REAGENT PREPARATION

In conducting the assay, follow strictly the test protocol. Room temperature incubation means: Incubation at 20 - 25°C.

Reagents with different lot numbers should not be mixed. The microtiter plate and all reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

The Calibrators **0 – 6** and **Control 1 and 2** are reconstituted with the **Dilution Buffer** provided in the Kit. It is recommended to keep the reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

Use the **Dilution Buffer** for the dilution of **Samples, Calibrators** and **Controls**.

The shelf life of the components after opening is not affected, if used appropriately. Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C. Reconstituted Components (**Calibrators 0 – 6** and **Control 1 and 2**) should be stored at -20°C (or below). Freezing extends the expiry at least 3 months. When using the calibrators anew, please thaw them rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay.

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for max. 4 weeks at 2-8°C.

Before use, all kit components should be brought to room temperature. **Precipitates, possible in buffers, should be dissolved before use through mixing and warming.**

The **Substrate Solution**, stabilised H₂O₂-Tetramethylbenzidine, is photosensitive – store and incubate in the dark.

When performing the assay, the Calibrators **0-6**, Control **1** and **2** and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times the Streptavidin HRP as well as the succeeding **Substrate Solution** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution** should be added to the plate in the same order as the Substrate Solution.

STORAGE CONDITIONS

The microtiter plate wells and all undiluted reagents are stable until the expiry date if stored in the dark at 2-8°C.

Store the unused seal strips and microtiter wells together with the desiccant at 2° to 8°C.

The Substrate Solution, stabilised H₂O₂-Tetramethylbenzidine, is photosensitive – store and incubate in the dark.

Reconstituted components should be stored at 2-8°C for up to 1 week. If longer storage time is needed, store the components frozen at -20°C or below. Freezing extends the expiry at least 2 months. Avoid repeated freeze-thaw cycles. In case you plan to perform multiple independent determinations over a longer period with one kit, you should aliquot the components prior to freezing into suitable smaller volumes. This is strongly recommended.

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only. For professional use only.

Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. DIAsource ImmunoAssays S.A. is not liable for any loss or harm caused by non-observance of the instructions, as far as no law withstands.

Temperature WILL affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

Do not use expired reagents.

Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

Caution: This kit contains material of human and/or animal origin.

Human Serum

Contained in following components: **Control Serum**

The sources of human sera were tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

Stop solution contains 0.2 M Sulfuric Acid (H₂SO₄)

R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

2-Methyl-4-Isothiazolin-3-one

contained in following components: **Biotin Conjugate, Streptavidin HRP, Dilution Buffer**

< 0.01% 2-Methyl-4-isothiazolin-3-one Solution

R34	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice

5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one

contained in following components: **Biotin Conjugate, Streptavidin HRP, Dilution Buffer, Washing Buffer**

< 0.01% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one

Solution

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water

General first aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes.

In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

ASSAY PROCEDURE

NOTES: All determinations (Calibrators, Control and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the Calibrators, Control Serum and the samples should be pipette as fast as possible (e.g., <15 minutes). To avoid distortions due to differences in incubation times, **Streptavidin HRP** as well as the following **Substrate Solution** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution** should be added to the plate in the same order as the Substrate Solution. Before beginning the test procedure bring all reagents to room temperature.

IMPORTANT: Please leave the wells A1/A2 until addition of the **Substrate Solution**, step 8, empty.

- 1) Please pipette in all needed wells, **except A1/A2**, **50 µl Biotine Conjugate**.
- 2) Pipette in positions B1/2 **50µl** each **Calibrator 0 (0 ng/ml)**,
pipette in positions C1/2 **50µl** each **Calibrator 1 (0.1 ng/ml)**,
pipette in positions D1/2 **50µl** each **Calibrator 2 (0.5ng/ml)**,
pipette in positions E1/2 **50µl** each **Calibrator 3 (1 ng/ml)**,
pipette in positions F1/2 **50µl** each **Calibrator 4 (2 ng/ml)**,
pipette in positions G1/2 **50µl** each **Calibrator 5 (4 ng/ml)**,
pipette in positions H1/2 **50µl** each **Calibrator 6 (8 ng/ml)**,

To control the correct accomplishment, **50 µl** of the **1:16** (or in respective dilution rate of the sample) in Dilution Buffer diluted **Control 1** and **2** can be pipetted in positions A3/4 and B3/4.

Pipette **50 µl each** of the **diluted samples** (generally 1:16 diluted in Dilution Buffer, please mix the dilutions immediately after sample addition and use within 60 minutes) in the rest of the wells, according to requirements.

- 3) Cover the wells with the sealing tape and incubate the plate for **1 hour at room temperature**
- 4) After incubation aspirate the contents of the wells and wash the wells **3 times** with **250 µl Washing Buffer**.
- 5) Following the last washing step, pipette **100 µl Streptavidin HRP** in each well, **except A1/A2**.
- 6) Cover the wells with the sealing tape and incubate **30 min at room temperature**
- 7) After incubation wash the wells 3 times with **Washing Buffer** as described in step 4)
- 8) Pipette **100 µl of the TMB-Substrate solution** in each well, **also in A1/A2**.
- 9) Incubate the plate for **15 Minutes in the dark at room temperature**.
- 10) After incubation pipette **100 µl Stop Solution** in each well **also in A1/A2**.
- 11) Measure the absorbance **within 30 minutes at 450 nm (Reference filter ≥590 nm, e.g. 620 nm)**.

CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.20, and the absorbance of Calibrator 6 should be greater than 1.00.

Samples, which yield higher absorbance values than **Calibrator 6**, are beyond the calibrator curve, for reliable determinations such samples should be retested at a higher dilution.

Establishing the Calibrator Curve

The calibrators provided contain the following concentration of native hIGFBP-1:

Calibrator	0	1	2	3	4	5	6
ng/ml	0	0.1	0.5	1	2	4	8

- 1) Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).
- 2) Subtract the mean absorbance of the blank from the mean absorbances of all other values.
- 3) Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis on semi-log paper.
- 4) Recommendation: Calculation of the calibrator curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **Non-linear regression, a higher-grade polynomial or four parametric logistic (4-PL)** curve fit usually are suitable for the evaluation.

- 5) The concentration in ng/ml of the samples can be calculated by multiplication with the respective dilution factor.

EXPECTATION VALUES

Concentrations of IGFBP-1 in human sera of 69 healthy adult donors were determined with the **DIAsource IGFBP-1 ELISA KAPME01**. Slight gender dependent differences were found, the concentrations of all samples varied from minimal 0.23 ng/ml to maximal 17.94 ng/ml (see table 5).

Table 5: Expectation values in sera of healthy adults (measured values in ng/ml)

Gender	No. of Samples	Average value	Median	Min. – Max.:
female	33	4.79	4.24	0.23 – 16.07
male	36	5.22	2.71	0.42 – 17.94
Total	69	5.01	2.77	0.23 – 17.94

REFERENCES

1. Rechler, M.M., *Insulin-like growth factor binding proteins*. Vitam Horm, 1993. **47**: p. 1-114.
2. Allander, S.V., et al., *Structure and chromosomal localization of human insulin-like growth factor-binding protein genes*. Growth Regul, 1993. **3**(1): p. 3-5.
3. Rutanen, E.M., et al., *Synthesis of placental protein 12 by human decidua*. Endocrinology, 1985. **116**(4): p. 1304-9.
4. Julkunen, M., et al., *Identification by hybridization histochemistry of human endometrial cells expressing mRNAs encoding a uterine beta-lactoglobulin homologue and insulin-like growth factor-binding protein-1*. Mol Endocrinol, 1990. **4**(5): p. 700-7.
5. Khosravi, J., et al., *Immunoassay of serine-phosphorylated isoform of insulin-like growth factor (IGF) binding protein (IGFBP)-1*. Clin Biochem, 2007. **40**(1-2): p. 86-93.
6. Westwood, M., *Role of insulin-like growth factor binding protein 1 in human pregnancy*. Rev Reprod, 1999. **4**(3): p. 160-7.
7. Rutanen, E.M., H. Bohn, and M. SeVPala, *Radioimmunoassay of placental protein 12: levels in amniotic fluid, cord blood, and serum of healthy adults, pregnant women, and patients with trophoblastic disease*. Am J Obstet Gynecol, 1982. **144**(4): p. 460-3.
8. Drop, S.L., et al., *Immunoassay of a somatomedin-binding protein from human amniotic fluid: levels in fetal, neonatal, and adult sera*. J Clin Endocrinol Metab, 1984. **59**(5): p. 908-15.
9. Hall, K., G. Lundin, and G. Povoia, *Serum levels of the low molecular weight form of insulin-like growth factor binding protein in healthy subjects and patients with growth hormone deficiency, acromegaly and anorexia nervosa*. Acta Endocrinol (Copenh), 1988. **118**(3): p. 321-6.
10. Busby, W.H., D.K. Snyder, and D.R. Clemmons, *Radioimmunoassay of a 26,000-dalton plasma insulin-like growth factor-binding protein: control by nutritional variables*. J Clin Endocrinol Metab, 1988. **67**(6): p. 1225-30.
11. Brismar, K., et al., *Insulin regulates the 35 kDa IGF binding protein in patients with diabetes mellitus*. J Endocrinol Invest, 1988. **11**(8): p. 599-602.
12. Suikkari, A.M., et al., *Prolonged exercise increases serum insulin-like growth factor-binding protein concentrations*. J Clin Endocrinol Metab, 1989. **68**(1): p. 141-4.
13. Maddux, B.A., et al., *IGF-binding protein-1 levels are related to insulin-mediated glucose disposal and are a potential serum marker of insulin resistance*. Diabetes Care, 2006. **29**(7): p. 1535-7.
14. Miell, J.P., et al., *The maternal insulin-like growth factor (IGF) and IGF-binding protein response to trisomic pregnancy during the first trimester: a possible diagnostic tool for trisomy 18 pregnancies*. J Clin Endocrinol Metab, 1997. **82**(1): p. 287-92.
15. Than, G.N., et al., *Levels of placenta-specific tissue protein 12 (VP12) in serum during normal pregnancy and in patients with trophoblastic tumour*. Arch Gynecol, 1983. **234**(1): p. 39-46.
16. Rutanen, E.M., F. Pekonen, and T. Karkkainen, *Measurement of insulin-like growth factor binding protein-1 in cervical/vaginal secretions: comparison with the ROM-check Membrane Immunoassay in the diagnosis of ruptured fetal membranes*. Clin Chim Acta, 1993. **214**(1): p. 73-81.
17. Martinez de Tejada, B., et al., *Can we improve the diagnosis of rupture of membranes? The value of insulin-like growth factor binding protein-1*. Bjog, 2006. **113**(9): p. 1096-9.

SUMMARY OF THE ASSAY PROCEDURE

Reconstitution / Dilution of Reagents		
Calibrators 0-6	Reconstitution in 500 µl Dilution Buffer	
Control 1 and 2	Reconstitution in 250 µl Dilution Buffer	
Wash Buffer	dilute in distilled water (eg. total volume of 50 ml in a graduated flask and fill up to 1000 ml)	1:20
Dilute Sample and Control 1 and 2 1:16 with Dilution Buffer		
Before beginning the test procedure bring all reagents to room temperature.		

Assay Procedure for Double Determinations:

Pipette	Reagent	Position
IMPORTANT: Leave the position A1 / A2 empty until addition of Substrate		
50 µl	Biotin Conjugate	In all wells except A1 / A2
50 µl	Calibrator 0 (0 ng/ml)	B1 and B2
50 µl	Calibrator 1 (0.1 ng/ml)	C1 and C2
50 µl	Calibrator 2 (0.5 ng/ml)	D1 and D2
50 µl	Calibrator 3 (1 ng/ml)	E1 and E2
50 µl	Calibrator 4 (2 ng/ml)	F1 and F2
50 µl	Calibrator 5 (4 ng/ml)	G1 and G2
50 µl	Calibrator 6 (8 ng/ml)	H1 and H2
50 µl	1:16 diluted Control 1	A3 and A4
50 µl	1:16 diluted Control 2	B3 and B4
50 µl	1:16 diluted Samples	following wells
Cover the wells with the sealing tape.		

Incubation: 1 h at RT, without shaking

3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer	each well
100 µl	Streptavidin HRP	each well, except A1/A2







Incubation: 30 min at RT, without shaking

3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer	each well
100 µl	Substrate	each well

Incubation: 15 min in the dark RT

100 µl	Stop Solution	each well
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.		

Revision date: 2011-06-24

	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
IVD	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 1251	Tracer
Ab 1251	Tracer
Ag 1251 CONC	Tracer concentrated
Ab 1251 CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoabsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
µP	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor