DRG

DRG® HCG ß free (EIA-4718)

CE

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1 INTRODUCTION

1.1 Intended Use

The **DRG Free β-HCG ELISA** is an enzyme immunoassay for the quantitative *in vitro* measurement of free beta subunit of human chorionic gonadotropin (free β-hCG) in serum. In the United States, this kit is intended for Research Use Only.

1.2 Summary and Explanation

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit.

2 PRINCIPLE OF THE TEST

The DRG Free β-HCG ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on a Free β-hCG molecule. An aliquot of patient sample containing endogenous Free β-hCG is incubated in the coated well with enzyme conjugate, which is an anti-β-HCG antibody [rabbit] conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of Free β-HCG in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Free β-HCG in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro use only. In the United States, this kit is intended for Research Use Only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.

- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG Instruments GmbH. The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti-β-HCG antibody (monoclonal).
2. ***Standard (Standard 0-5)***, 6 vials (lyophilized), 1.0 mL;
   Concentrations: 0 – 10.0 – 25.0 – 50.0 – 100.0 – 200.0 ng/mL;
   The concentrations of the DRG Free β-HCG Kit standards match the WHO Reference Reagent Human Chorionic Gonadotrophin, Beta Subunit (Purified) (NIBSC code: 99/650)
   See „Preparation of Reagents".
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
3. ***Control (Low and high)***, 2 vial (lyophilized), 1.0 mL,
   see „ Preparation of Reagents "
   For control values and ranges please refer to vial label or QC-Datasheet.
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
4. ***Zero Buffer***, 1 vial, 14 mL, ready to use,
   * contains 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
5. ***Enzyme Conjugate***, 1 vial, 18 mL, ready to use,
   Anti β-HCG antibody conjugated to horseradish peroxidase;
6. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
7. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
8. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents".

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Zero Buffer* for sample dilution is available upon request.

4.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or Deionized water
- Timer (60 min. range).
- semi-logarithmic graph paper or software for data reduction

4.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vials with 1.0 mL Aqua dest. and let stand for 10 minutes in minimum. Mix several times before use.

Note: *The reconstituted standards are stable for up to 30 days at 2-8°C.*

Controls

Reconstitute the lyophilized content of the control vials with 1.0 mL Aqua dest. and let stand for 10 minutes in minimum. Mix several times before use.

Note: *The reconstituted controls are stable for up to 30 days at 2-8°C.*

Wash Solution

Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.

The diluted Wash Solution is stable for 2 weeks at room temperature.

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN

Serum should be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying.

Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Zero Buffer* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL Serum + 90 µL *Zero Buffer* (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Zero Buffer* (mix thoroughly).

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** ***Zero Buffer*** into each well.
   Thoroughly mix for 30 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at 37 °C.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense **150 µL** ***Enzyme Conjugate*** into each well.
7. Incubate for **30 minutes** at 37 °C.
8. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **100 µL** of ***Substrate Solution*** to each well.
10. Incubate for **20 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
    It is important to make sure that all the blue color changes to yellow color completely.
12. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 15 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard		Optical Units (450 nm)
Standard 0	(0 ng/mL)	0.02
Standard 1	(10.0 ng/mL)	0.22
Standard 2	(25.0 ng/mL)	0.46
Standard 3	(50.0 ng/mL)	0.81
Standard 4	(100.0 ng/mL)	1.28
Standard 5	(200.0 ng/mL)	1.97

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

1. Free β-hCG Subunit Levels in Normal Pregnancy

238 samples of pregnant women in the 1st trimester have been measured with the DRG Free β-HCG ELISA.

week of gestation	day of gestation	Median (sst) [ng/mL] weight independent	Median (f) [ng/mL] weight 50 kg	Median (f) [ng/mL] weight 65 kg	Median (f) [ng/mL] weight 100 kg	Median of week [ng/mL]
8	59	139.6	159.9	144.9	115.2	130.4
9	66	120.2	137.4	124.5	99.0	155.1
10	73	103.5	118.0	107.0	85.1	98.2
11	80	89.1	101.4	91.9	73.1	91.8
12	87	76.7	87.1	79.0	62.8	75.8
13	94	66.0	74.9	67.9	54.0	62.3

2. hCG and Free Subunits Levels in Gestational Choriocarcinoma

Free α and free β-subunits and hCG levels were measured in five patients with untreated gestational choriocarcinoma. The concentrations in serum are shown in the following table.

Patient Number	hCG (ng/mL)	Free α-hCG (ng/mL)	Free β-hCG (ng/mL)
1	210,000	112	8,000
2	22,195	20	1,300
3	6,840	1	232
4	36,000	44	3,900
5	4,200	2	350

The levels of free α-hCG were low, ranging from 1-112 ng/mL, whereas hCG levels ranged from 4,200 to 210,000 ng/mL (1 ng ≈ 15 mIU). In contrast, free β-hCG concentrations were found to be markedly elevated in choriocarcinoma.

3. Ectopic Production of hCG and Free Subunits by Nontrophoblastic Tumors

The following table shows results obtained in various tumors and healthy and benign disease controls:

Measurement of hCG, α-hCG, and β-hCG serum levels in nontrophoblastic tumors, benign disease, and healthy controls

Tumor type	No. of samples	hCG (ng/mL)	α-hCG (ng/mL)	β-hCG (ng/mL)
Cervix	20	0	1 (1.6)[a]	1 (0.65)
Corpus uterus	20	0	0	0
Gastric	20	0	0	1 (1.5)
Pancreatic	20	0	1 (16.0)	2 (0.8, 3.1)
Colon	20	0	0	0
Lung	20	0	1 (90.0)	1 (0.7)
Ovarian	20	0	1 (1.8)	0
Prostate	20	0	1 (1.6)	0
Other digestive tract tumor	18	0	0	0
Total [%]	178	0	5 [3]	5 [3]
Benign disease controls	61	0	1 (1.6)	0
Healthy controls	50	0	0	0
Total [%]	111	0	1 [1]	0

[a] The number in parentheses represents the measured value in ng/mL.

The cut-off values for positive results are 1.5 ng/mL for hCG and α-hCG and 0.4 ng/mL for β-hCG.

When compared with healthy control values, all nontrophoblastic cancer patients had hCG concentration within the normal range (~ 0.9 ng/mL). Free subunits were elevated in 10 of 178 patients. It is noteworthy that α-hCG levels in two patients (pancreatic and lung tumors) were relatively high (16 and 90 ng/mL, respectively), whereas the maximum concentration of free β-hCG was only 3.1 ng/mL (pancreatic tumor).

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 ASSAY CHARACTERISTICS

9.1 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Hormone tested	Concentration	Produced Color Intensity Equivalent to free ß-HCG in Serum
TSH	25 µIU/mL	< 0.3 ng/mL
FSH	100 mIU/mL	< 0.2 ng/mL
Prolactin	100 mIU/mL	< 0.5 ng/mL
LH	200 mIU/mL	< 1 ng/mL

9.2 Analytical Sensitivity

The analytical sensitivity was found to be 0.2 ng/mL.

9.3 Precision

9.3.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	8.73	6.05
2	20	17.83	3.79

9.3.2 Inter Assay Variation

The between assay variability (three different kit lots) is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	7.39	4.15
2	12	16.22	5.88

9.4 Recovery

Recovery of the DRG ELISA was determined by adding increasing amounts of the analyte to two different patient sera containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2
Concentration [ng/mL]		7.84	13.79
Average Recovery [%]		99.6	93.5
Range of Recovery [%]	from	89.6	86.2
	to	106.3	98.3

9.5 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		7.84	13.79	29.55
Average Recovery [%]		106.8	102.4	96.0
Range of Recovery [%]	from	98.0	93.2	89.3
	to	111.5	111.0	104.9

10 LIMITATIONS OF USE

Any improper handling of samples or modification of this test might influence the results.

10.1 High-Dose-Hook Effect

No hook effect was observed in this test up to 200 ng/mL of Free β-HCG.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

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SYMBOLS USED WITH DRG ELISAS

Symbol	English	Deutsch	Français	Español	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
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.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	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 conservacion	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	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´ arresto
Zero Standard	Zero Standard	Nullstandard	Zero Standard	Estándar cero	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
1 N HCl	1 N HCl	1 N HCl	1N HCl	1 N HCl	
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni