DRG® HCG ELISA (EIA-1469)

USA: RUO

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Introduction

INTENDED USE

The **DRG HCG ELISA** is an enzyme immunoassay for the measurement of intact human chorionic gonadotropin (hCG) in serum or plasma. In the United States, this kit is intended for Research Use Only.

SUMMARY AND EXPLANATION

Chorionic Gonadotropin (hCG) is a glycoprotein hormone which is normally produced by the placenta during pregnancy. After conception, the hCG concentration increases rapidly to reach a peak near the end of the first trimester. High concentrations are observed throughout pregnancy. After delivery, hCG levels fall rapidly and become undetectable after a few days.

Structurally intact hCG molecules are composed of an alpha and a beta subunit with a molecular weight of 38.4 kDa. The alpha subunit is nearly identical to the alpha subunits of other glycoprotein hormones, such as Thyroid Stimulating Hormone (TSH), Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH): The differences in the beta subunit of the respective hormones account for their biological specificity and immunochemical distinctiveness.

Monoclonal antibodies recognizing unique sites on the beta chain of the hCG molecule are essential for differentiation between hCG and LH, FSH and TSH.

HCG Assays are used for the early detection of pregnancy.

1. In addition to the elevated hCG levels during pregnancy, high concentrations of hCG may be associated with neoplasms of trophoblastic and nontrophoblastic origin such as hydatiform mole, chorionepithelioma, embryonal cell carcinoma, and many others.
2. HCG is commonly elevated in different testicular tumors and is thus used as a tumor marker for testicular tumors in combination with AFP. There is a good correlation between changes in hCG levels and response to therapy.
3. Extragonadal germ-cell cancers in the absence of clinically or ultrasonographically detectable testicular abnormalities have beein observed as well. Over 50% of patients with malignant insulinomas have elevated hCG levels: the hormone is not detected in association with benign adenomas. Ectopic secretion of hCG also have been found in a small percentage of patients with adenocarcinoma of the ovary, pancreas and stomach, hepatomas, and islet-cell carcinomas.

PRINCIPLE of the test

The DRG 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 hCG molecule. An aliquot of sample containing endogenous hCG is incubated in the coated well with enzyme conjugate, which is a monoclonal antibody directed against the alpha-chain of hCG conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of hCG in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of hCG in the donor sample.

Warnings and Precautions

1. This kit is for in vitro use only. For professional use only.
2. 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.
3. 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.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21°C to 26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the donor samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. 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.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

Reagents

REAGENTS PROVIDED

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti-β-HCG antibody (monoclonal).
2. ***Standard (Standard 1-5)***, 5 vials (lyophilized), 1.0 mL;
   Concentrations: 5; 50; 200; 500; 1000 mIU/mL
   Conversion: 1 pg/mL = 0,00916 mIU/mL
   The concentrations of the DRG HCG Kit standards match the international Reference material Chorionic Gonadotropin, Reference reagent 2001 (NIBSC Code number 99/688).
   See „Preparation of Reagents“;
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
3. ***Sample Diluent***, 1 vial, 10 mL, ready to use,
   0 mIU/mL
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
4. ***Enzyme Conjugate***, 1 vial, 11 mL, ready to use,
   Monoclonal antibody against the alpha-subunit conjugated to horseradish peroxidase;
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
5. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
6. ***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.

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

Note: Additional *Sample Diluent* for sample dilution is available upon request.

MATERIALS REQUIRED BUT NOT PROVIDED

1. A microtiter plate calibrated reader (450±10 nm) (e.g. the DRG International, Inc. Microtiter Plate Reader).
2. Calibrated variable precision micropipettes.
3. Absorbent paper.
4. Distilled or deionized water
5. Timer
6. Semi logarithmic graph paper or software for data reduction

STORAGE CONDITIONS

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

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REAGENT PREPARATION

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

Standards

Reconstitute the lyophilized contents of the standard vial with 1.0 mL Aqua dest.
Note: *The reconstituted standards are stable for 2 months at 2 °C - 8 °C. For longer storage freeze at -20°C.*

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.

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.

SPECIMEN Collection and Preparation

Serum or plasma (EDTA-, Heparin- or citrat plasma) can 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.

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. Donors receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001;
for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

SPECIMEN STORAGE AND PREPARATION

Specimens should be capped and may be stored for up to 5 days at 2 °C - 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.

SPECIMEN DILUTION

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* 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 *Sample Diluent* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly).

NOTE: Sera of pregnant women must be diluted 1/100 in *Sample Diluent* before starting the assay.

Assay procedure

GENERAL REMARKS

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
4. 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.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.

TEST PROCEDURE (QUANTITATIVE METHOD)

Each run must include a standard curve.

NOTE: Sera of pregnant women must be diluted 1/100 in *Sample Diluent* before starting the assay. (s. 5.3.)

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **25 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense **100 µL** *Enzyme Conjugate* into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with distilled water (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. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
9. 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 10 minutes** after adding the *Stop Solution.*

TEST PROCEDURE (QUALITATIVE METHOD)

This procedure differentiates positive (pregnant) from negative samples by comparing the sample hCG levels with *Sample Diluent* (0 mIU/mL) and *Standard 2(* 50 mIU/mL).
Donor samples are run in parallel with the *Sample Diluent* (0 mIU/mL) and the 50 mIU/mL *Standard 2*. The assay procedure is identical with the Quantitative Method, but step 9 and 10 is omitted.

CALCULATION OF RESULTS (QUANTITATIVE)

1. Calculate the average absorbance values for each set of standards, controls and donor samples.
2. Using semi-logarithmic graph paper, 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 or reported as > 1000 mIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

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 1 (5 mIU/mL)	0.05
Standard 2 (50 mIU/mL)	0.14
Standard 3 (200 mIU/mL)	0.43
Standard 4 (500 mIU/mL)	0.94
Standard 5 (1000 mIU/mL)	1.54

QUALITATIVE RESULTS

For a qualitative analysis of the hCG level the color development of the specimen is compared with the color of the *Sample Diluent* (0 mIU/mL) and *Standard 2* (50 mIU/mL).
If the blue color is less intense than the color of the 50 mIU/mL *Standard*, the sample is considered as negative.
If the blue color is more intense than or equal to the color of the 50 mIU/mL *Standard* the sample is considered as positive.

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 donor 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.

Performance Characteristics

ASSAY DYNAMIC RANGE

The range of the assay is between 5 – 1000 mIU/mL.

SPECIFICITY OF ANTIBODIES (CROSS REACTIVITY)

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

Protein	Concentration	Produced Colour Intensity Equivalent to HCG in serum (mIU/ml)
hLH	300 mIU/mL	9
hLH	200 mIU/mL	< 5
hLH	80 mIU/mL	< 5
TSH	75 µIU/mL	10
TSH	50 µIU/mL	6
TSH	25 µIU/mL	< 5
FSH	200 mIU/mL	< 5
FSH	50 mIU/mL	< 5

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

INTERFERING SUBSTANCES

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

DRUG INTERFERENCES

Until today no substances (drugs) are known to us, which have an influence to the measurement of hCG in a sample.

HIGH-DOSE-HOOK EFFECT

No hook effect was observed in this test up to 13,300 mIU/mL of hCG.

Legal Aspects

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.

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.

REFERENCES

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