HUMAN CHORIONIC GONADOTROPIN (hCG) ENZYME IMMUNOASSAY TEST KIT Catalog Number: 6107310

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Enzyme Immunoassay for the Quantitative Determination of Human Chorionic Gonadotropin (hCG) Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

hCG Enzyme Immunoassay

INTENDED USE

For the quantitative determination of Human Chorionic Gonadotropin (hCG) concentration in human serum.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally produced by the placenta during pregnancy. The hCG molecule consists of two combined, dissimilar subunits designated alpha and beta. The beta subunit, with a molecular weight of approximately 30,000 daltons, confers biological and immunological specificity to the entire hCG molecule by virtue of its unique amino acid sequence and content. The alpha subunit, with a molecular weight of approximately 18,000 daltons, is essentially identical to the alpha subunit of the pituitary glycoprotein hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH).

The appearance of hCG in urine or serum soon after conception and its rapid rise in concentration makes it an ideal indicator for the detection and confirmation of pregnancy. However, elevated hCG levels are also frequently associated with trophoblastic and non-trophoblastic neoplasmas; these conditions should be considered before a diagnosis of pregnancy can be made.

Immunoassays utilizing antibodies specific to the beta subunit of hCG provide a sensitive and specific technique allowing early detection of pregnancy around the time of the first missed menstrual period.

In women with a multiple pregnancy (twins, triplets, etc.), levels of hCG have been reported to be higher than those expected during a normal single pregnancy. This is probably the result of the increased placental mass necessary to sustain multiple fetuses. Also, as one might suspect, cases of placental insufficiency show levels of hCG lower than those expected during normal pregnancy. Decreased values have also been associated with threatened abortion and ectopic pregnancy.

Principle of the Test

The β-HCG ELISA TEST is based on simoultaneous binding of human b-HCG to two monoclonal antibodies, one immobilized on microwell plates, the other conjugated with horseradish peroxidase (HPR).
After incubation the bound/free separation is performed by a simple solid-phase washing, then the substrate solution (TMB) is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance are determinated.
The b-HCG concentration in the sample is calculated based on a series of standard.
The color intensity is proportional to the b-HCG concentration in the sample.
The ELISA test is performed as an indirect solid phase sandwich-type immunoassay. Microwells are coated with anti-monoclonal β - HCG followed by blocking the unreacted sites to reduce non-specific binding.

Step 1 β - hCG Antigens present in calibrators and patient samples bind to the coated antibody.

Step 2 The Antigen-Antibody complex is reacted with enzyme (HRP) labeled anti-monoclonal β - HCG conjugate resulting in the β - HCG antigen being sandwiched between the solid phase antibody and the enzyme conjugate.

Step 3 The enzyme converts added substrate (TMB) to form a colored solution.

Step 4 The intensity of color change, which is proportional to the concentration of Antibodies present in the samples is read by a microplate-reader at 450 nm. Results are expressed in mIU/ml.

REAGENTS

Materials provided with the kit:

- Microwell plate. 12x8 well strips. Individually separable wells. Coated with anti-monoclonal β –HCG., packaged in an aluminum bag with a drying agent.
- Standards. 5 Vials. Standard A – Standard E each 0.4 mL, ready to use. Concentrations 0; 5; 20; 75 and 200 mIU/mL
- Sample diluent. 1 vial of Sample diluent 10 mL.
- Enzyme Conjugate. anti-β–HCG HRP-horseradish peroxidase (HRP). Ready-to-use. 12 mL.
- TMB Substrate solution. H_2O_2-TMB 0.25 g/L (avoid any skin contact). 12 mL.
- Stop Solution. Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL.
- Washing buffer. 1 bottle 25 mL, 40 fold.

Materials required but not provided:

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- Multichannel pipettes and micropipettes (Precision ≥1.5%) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.
The serum or plasma β - HCG values are comprised in the following intervals:

Sample	Range mIU/mL
not pregnant woman	< 8.0 mIU/ml
Pregnancy	
1-3th week	0 – 50 mIU/ml
4th week	0– 400 mIU/ml
5th week	280 – 19400 mIU/ml
6th week	2030 – 49700 mIU/ml
2nd month	18900 – 133400 mIU/ml
3nd month	25530 – 229600 mIU/ml

STORAGE OF TEST KITS

The components will remain stable through the expiration date shown on the label if stored between 2-8°C in dark. Do not frezee. Do not use reagents beyond the kit expiration date.
The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells.
Once opened the bag, microplate strips are stable for 1 month at 2-8°C in the plastic bag tightly sealed, with the silicagel.
Opened reagents are stable for 1 month at 2-8°C.

REAGENT PREPARATION

- Coated microwell strips are for one time use only.
- Calibrators, Substrate Solution, Enzyme Conjugate and Stop Solution are ready to use and need not to be diluted.
- Washing Buffer is concentrated and need to be diluted.

PRECAUTIONS

- Instructions should be followed exactly as they appear in this kit insert to ensure valid results.
- Avoid contact with the TMB (3,3`,5,5`-Tetramethyl-benzidine). If TMB comes into contact with skin wash thoroughly with water and soap.
- The stop solution contains sulphuric acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
- Avoid contact between the buffered peroxide solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.
- Do not use beyond expiration date on the label.
- Do not use if reagent is not clear or if a precipitate is present.
- Do not interchange kit components with those from other sources other than the same catalog number from DIMA.
- Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling.
- All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials.

SPECIMEN COLLECTION AND PREPARATION

Only Serum or Plasma specimens should be used in this procedure. The patients need not to be fasting, and no special preparations are necessary.

Use fresh serum or plasma. Samples can be stored at 2-8°C for 2 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing.

Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Neither Bilirubin nor Hemolysis have significant effect on the procedure.

Store specimens at 2°- 8°C for up to a maximum of 2 days. For longer storage, specimens should be frozen. Avoid repeated freezing and thawing of samples. For sample with concentration over 200 mIU/mL dilute the sample with Sample Diluent.

ASSAY PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.

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- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.
- Good washing technique is critical. For manual washing, fill each microwell with 200-300 µL Washing Buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.

Preparation of Reagents

Washing Buffer	Add 25 mL Washing Buffer to 975 mL of distilled water.

Preparation of sample

Dilute samples with concentrations above 200 mIU/mL with sample Diluent.

Dilute samples from woman after the 4th pregnancy week 1:50 with sample Diluent.

Test Procedure

Label protocol sheet to indicate sample placement in the wells according to the following figure. 5 calibrators (standards) (A-E) and 1 Blank should be included. The user has the option to run Patient Samples (P) in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
a	Blank	D	P3									
b	A	E	P4									
c	A	E	P4									
d	B	P1	P..									
e	B	P1	P..									
f	C	P2										
g	C	P2										
h	D	P3										

Cal.	Conc. mIU/mL
A	0
B	5
C	20
D	75
E	200

1. Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder .
2. Pipette 25 µL of Calibrators and Patient Samples into the wells.
3. Seal the plate and incubate 20 minutes at 37° C.
4. Add 100 µL of Enzyme Conjugate to the wells except for Blank well seal the plate and incubate 60 minutes at 37°C.
5. Discard the contents of the microwells and wash the wells with 200-300 µL Washing buffer.

   Repeat the washing procedure for 4 times.
6. Pipette 100 µL of Substrate Solution into each microwell in the same order and timing as for the Enzyme Conjugate, Blank well included.
7. Incubate 12 minutes at room temperature.
8. Add 100 µL of Stop Solution into each microwell using the same order and timing as for the addition of the Substrate Solution.
9. Read absorbance of each microwell at 450 nm against blank using a microplate reader.

TEST EVALUATION

Mean absorbance and relative percentage

1. Calculate the mean of the absorbances (Em) corresponding to the single points to the standard curve and of each sample
2. Subtract the mean absorbance value of the zero standard from the mean absorbance values of standards and samples.
3. Draw the standard curve on log-log graph paper by plotting absorbance values of standard against appropriate β - hCG concentration.
4. Read off the β - hCG concentrations of the calibrators and samples.

LIMITATIONS OF THE PROCEDURE

The assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only.

PERFORMANCE CHARACTERISTICS

Sensitivity
The minimal detectable concentration of β - hCG by this assay is estimated to be 1 mIU/ml.

Specificity
The cross reaction of the coated microplate calculated according are shown in the table:

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Telf. (+34) 934 694 990 Fax. (+34) 934 693 435. website www.linear.es

β - hCG	100.0 %
α - HCG	0.3 %
hLH	0.01 %
hFSH	0.01 %
hTSH	0.01 %

Precision

a. Intra Assay variation

Within-run precision was determined by replicate determination of three different control in one assay. The within assay variability is shown below:

Sample	1	2	3
Number of replicates	7	7	7
Mean β –HCG (mIU/mL)	161	57	24,5
Std. Deviation	7	2.5	1.2
Coef. of Variation (%)	4.35	4.03	4.72

b. Inter Assay variation

Between-run precision was determined by replicate determination of three different controls in one assay. The between assay variability is shown below:

Sample	1	2	3
Number of replicates	16	16	16
Mean β - hCG (mIU/mL)	1.4	28.4	65.4
Std. Variation	0.08	1.75	2.83
Coef. of Variation (%)	5.7	6.16	4.32

Recovery

Various patient samples of known β - hCG levels were combined and assayed in duplicate. The average recovery 99.5 % with reference to the original concentrations.

Expected conc.	Observed conc.	Recovery
18.8	18.4	97.8
41.4	43.3	104.5
76.8	73.2	95.3

Linearity

Two patient samples were serially diluted with sample diluent in a linearity study. The average recovery was 102.7 %.

Patient	Exp. Conc	Obs. Conc	Recovery
1		62.8	
Dil. 1 / 2	31.4	33.1	105.4
Dil. 1 / 4	15.7	16.1	102.5
Dil. 1 / 8	7.8	8.4	107.6
2		12.8	
Dil. 1 / 2	6.4	6.5	101.6
Dil. 1 / 4	3.2	3.0	93.7
Dil. 1 / 8	1.6	1.7	106.1

Limitations of the procedure

In this assay, no hook effect is observed up to 10.000 mIU/mL of β - HCG.

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LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN
Telf. (+34) 934 694 990 Fax. (+34) 934 693 435. website www.linear.es