Human HCG - free ELISA Kit

Cat. No.:DEIA2027
Pkg.Size:96T

Intended use

This product is an enzyme immunoassay for measurement of free β- subunit of human chorionic gonadotropin (free β-hCG) in serum.

General Description

In molecular biology, human chorionic gonadotropin (hCG) is a hormone produced during pregnancy that is made by the developing placenta after conception, and later by the placental component syncytiotrophoblast. Some cancerous tumors produce this hormone; therefore, elevated levels measured when the patient is not pregnant can lead to a cancer diagnosis. However, it is not known whether this production is a contributing cause or an effect of tumorigenesis. The pituitary analogue of hCG, known as luteinizing hormone (LH), is produced in the pituitary gland of males and females of all ages. As of December 6, 2011 (2011 -12-06)[update], the FDA has prohibited the sale of "homeopathic" and OTC hCG diet products and declared them fraudulent and illegal.

Principle Of The Test

The 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 specimen 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 specimen sample.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (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; Contain non-mercury preservative.
3. Control (Low and high), 2 vial (lyophilized), 1.0 mL, For control values and ranges please refer to vial label or QC-Datasheet. Contain non-mercury preservative.
4. Zero Buffer, 1 vial, 14 mL, ready to use, Contains non-mercury 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 0.5 M H2SO4, Avoid contact with the stop solution. It may cause skin irritations and burns.
8. Wash Solution, 1 vial, 30 mL (40X concentrated);

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 ± 10 nm).
2. Calibrated variable precision micropipettes.
3. An incubator suitable for incubation 37 ℃

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4. Absorbent paper.
5. Distilled or Deionized water
6. Timer (60 min. range).
7. Linear graph paper or software for data reduction

Storage

Store all contents at 2 to 8 ℃.

Specimen Collection And Handling

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.
1. Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.
2. Specimen Storage and Preparation: Specimens should be capped and may be stored for up to 24 hours at 2 ℃ - 8 ℃ prior to assaying. Specimens held for a longer time should be frozen only once at -20 ℃ prior to assay. Thawed samples should be inverted several times prior to testing.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.
1. Standards: Reconstitute the lyophilized contents of the standard vials with 1.0 mL distilled water and let stand for 10 minutes in minimum. Mix several times before use.
2. Controls: Reconstitute the lyophilized content of the control vials with 1.0 mL distilled water and let stand for 10 minutes in minimum. Mix several times before use.
3. Wash Solution: Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
4. 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.

Assay Steps

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 ℃.
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 ℃.
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

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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.

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

Calculation

1. Calculate the average absorbance values for each set of standards, controls and specimen 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 such. For the calculation of the concentrations this dilution factor has to be taken into account.

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

Detection Range

2.5-50 ng/mL

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Sensitivity

0.196 ng/mL

Precautions

1. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
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 ℃ to 8 ℃ 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 ℃ – 26 ℃) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the specimen 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.

Analyte Gene Information

Gene Name	CGB chorionic gonadotropin, beta polypeptide [Homo sapiens]
Official Symbol	CGB

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Synonyms	CGB; chorionic gonadotropin, beta polypeptide; choriogonadotropin subunit beta; CGB3; CG-beta; chorionic gonadotropin beta chain; chorionic gonadotrophin chain beta; chorionic gonadotropin beta subunit; chorionic gonadotropin beta 3 subunit; CGB5; CGB7; CGB8; hCGB;
GeneID	1082
mRNA Refseq	NM_000737
Protein Refseq	NP_000728
MIM	118860
UniProt ID	P01233
Chromosome Location	19q13.3
Pathway	Glycoprotein hormones, organism-specific biosystem; Metabolism, organism-specific biosystem; Metabolism of amino acids and derivatives, organism-specific biosystem; Peptide hormone biosynthesis, organism-specific biosystem;
Function	hormone activity;

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