β-HCG (Human Chorionic Gonadotropin) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAHCG1	βHCG Elisa	96 Tests

Intended Use:

B HCG ELISA is intended for quantitative measurement of Human Chorionic Gonadotropin in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Human chorionic gonadotropin concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by placental tissue, beginning with the primitive trophoblast, almost from the time of implantation and serves to support the corpus luteum during the early weeks of pregnancy. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and diagnosis of early pregnancy disorders.

According to the literature, hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/ml by the first missed period. At the time for the next ovulation, the hCG level is 200 mIU/ml (approximately 28 days after conception). A peak of 50,000 or even 100,000 mIU/ml is attained by the third month, then a gradual decline is observed.

The hCG ELISA is based on a sandwich ELISA principle. The assay system utilizes one anti-hCG antibody for solid phase immobilization and another monoclonal anti-hCG antibody conjugated to horseradish peroxidase. Samples are incubated on the plate during which any hCG in the sample interacts with the coated antibody and is bound to the plate surface. The wells are washed to remove excess sample then conjugate is added. The second, labelled antibody also binds to the hCG, and after a wash step to remove unbound antibody, a solution of TMB is added which reacts with the label resulting in the development of a blue colour. The addition of acid stop solution stops the reaction, the colour is changed from blue to yellow and the absorbance measured at 450nm. The concentration of hCG is directly proportional to the intensity of colour developed.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	One 96 well microplate coated with beta hCG and packaged in an aluminium bag with a drying agent. Store at 2-8°C.
hCG Calibrators	6x1ml	6 vials of references for hCG antigens at levels of 0.0, 5.0, 20.0, 50.0, 150, 300 mIU/ml. Store at 2-8°C. The calibrators were standardized against a reference preparation which was assayed against the WHO 3rd IS (75/537).
hCG Enzymatic Reagent	1x18ml	1 vial containing enzyme labelled affinity purified antibody to HCG, in buffer, dye and preservative. Store at 2-8°C.
Zero Buffer	1x12ml	1 vial containing buffered saline. Store at 2-8°C.
Wash Solution Concentrate (50x)	1x15ml	One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
TMB Substrate	1x12ml	One bottle containing TMB in buffer. Store at 2-8°C.
Stop Solution	1x12ml	One bottle containing a strong acid 1N HCl. Store at 2-30°C.

Plastic Sealable bag, IFU and plate covers.

NOTE 1: Do not use reagents beyond the kit expiration date.

NOTE 2: Avoid extended exposure to heat and light. Opened reagents are stable for 60 days when stored at 2-8°C. Kit and component stability are identified on the label.

NOTE 3: Above reagents are for single 96 well microplate.

Materials provided but not required:

Distilled water, Timer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection, Storage and Stability:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Blood should be collected in plain red top venepuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, the samples may be stored in temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml of the specimen is required.

Quality Control:

Each laboratory should assay controls at levels in low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality Control charts should be maintained to follow the performance of the supplied reagents. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

Reagent preparation:

1. **Wash Buffer**: 1 volume of wash buffer (50x) with 49 volumes of distilled water. Dilute 15ml of wash buffer into distilled water to prepare 750ml of total wash buffer. Mix well before use.

TEST PROCEDURE:

Before proceeding with the assay, being all reagents, serum references and controls to room temperature (18-22°C)

STEP 1

Preparation: Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminium bag, seal and store at 2-8°C.

STEP 2

Addition of Samples and calibrators: Add 50 µl of the calibrators and samples and controls into appropriate wells.

STEP 3

Addition of Zero Buffer: Add 100 µl of Zero Buffer to all wells.

STEP 4

Incubation: Tap the microplate gently for 20-30 seconds to mix and cover the plate with the plate cover and incubate for 30 minutes at room temperature.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of Enzyme Conjugate: Add 150 µl of Enzyme Conjugate into each well. Gently mix for 5 seconds

STEP 7

Incubation: Incubate for 30 minutes at room temperature.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover and the contents of the microwell. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of the Substrate: Add 100 µl of the TMB Substrate Solution to each well. Always add reagents in the same order to minimize reaction time differences between wells. Incubate at room temperature for 20 minutes. Ensure this incubation takes place in the dark.

STEP 10

Stopping the Reaction: Add 100 µl of the Stop solution into each well and mix gently. Shake the plate gently for 15-20 seconds to mix, till the solution changes to yellow from blue.

STEP 11

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances. Read the results within 30 minutes of addition of the stop solution.

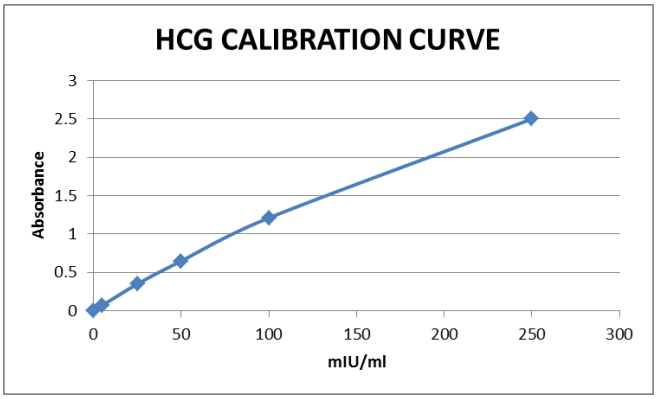
Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in mIU/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.063	0.0 mIU/ml
CAL B	0.120	5 mIU/ml
CAL C	0.581	20 mIU/ml
CAL D	1.269	50 mIU/ml
CAL E	1.503	150 mIU/ml
CAL F	2.624	300 mIU/ml

Procedure:



Notes:

1. The MSDS and Risk Analysis form for this product is available on request.
2. It is important that the time of reaction in each well is held constant to achieve reproducible results.
3. Pipetting of samples should not be extended beyond 10 minutes to avoid assay test drift.
4. Highly lipaemic, haemolysed or grossly contaminated specimens should not be used.
5. If more than one plate is used, it is recommended that the dose response curve is repeated.
6. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
7. Plate reads measure vertically. Do not touch the bottom of the wells.
8. Failure to remove adhering solution adequately in the aspiration or decantation wash steps may result in poor replication and spurious results.
9. Use components from the same lot. No intermixing of reagents from different batches.
10. Patient specimens with hCG concentrations above 250 mIU/ml may be diluted with normal male serum (hCG <1 mIU/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
11. Accurate and precise pipetting as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this IFU may yield inaccurate results.
12. All applicable national standards, regulations and laws including, but not limited to, good laboratory procedures must be strictly followed to ensure compliance and proper device usage.
13. It is important that the user calibrate all equipment in use and to perform routine preventive maintenance.

Interpretation:

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Prestige Diagnostics has no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. False positive results may occur in the presence of a wide variety of trophoblastic and nontrophoblastic tumours that secrete hCG. Therefore, the possibility of an hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.
7. Also, false positive results may be seen when assaying specimens from individuals taking the drugs Pregonal and Clomid. Additionally Pregonal will often be followed with an injection of hCG.
8. Spontaneous microabortions and ectopic pregnancies will tend to have values which are lower than expected during a normal pregnancy while somewhat higher values are often seen in multiple pregnancies.
9. Following therapeutic abortion, detectable hCG may persist for as long as three to four weeks. The disappearance rate of hCG after spontaneous abortions will vary depending upon the quantity of the residual trophoblast.
10. A hCG value alone is not of diagnostic value and should only be used in conjunction with other clinical manifestations and diagnostic procedures.

Expected Ranges of Values:

hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of hCG in the serum of pregnant women increases to 5-50mIU/ml within one week after implantation and continues increasing exponentially during the first ten weeks reaching a maximum of 100,000 – 200,000 mIU/ml at the end of the first trimester.
Laboratories must measure an in-house range for the indigenous population.

Performance Characteristics:

Precision:
The within and between assay precisions of hCG Elisa were determined by analyses on 3 different levels of control sera. The details are provided below:

Within run assay precision:

SAMPLE	N	MEAN	SD	CV
Level 1	20	4.4	0.22	4.9%
Level 2	20	18.7	0.75	4.0%
Level 3	20	214.8	14.59	6.8%

Between run assay precision:

SAMPLE	N	MEAN	SD	CV
Level 1	20	5.4	0.52	9.6%
Level 2	20	22.4	1.97	8.8%
Level 3	20	213.1	15.16	7.1%

Sensitivity:
The hCG Elisa test system has a sensitivity of 2.0 mIU/ml. The analytical sensitivity was ascertained by determining the variability of the 0 calibrator and using the 2SD statistic to calculate the minimum dose.

Accuracy:
The hCG Elisa was compared to a Radio Immunoassay. Biological specimens from normal and pregnant populations were assayed. Below are the details.

METHOD	MEAN	LEAST SQUARE REGRESSION	CORRELATION COEFFICIENT
THIS METHOD	14.8	Y=0.081 + 0.93 (x)	0.989
REFERENCE	15.1		

Specificity:
The cross-reactivity of the hCG Elisa to selected substances was evaluated by adding the interfering substances to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance of Chorionic gonadotropin needed to produce the same substance.

Substance	Cross Reactivity	Concentration
HCG	1.000	-
Beta subunit	<0.0001	1000 ng/ml
FSH	<0.0001	1000 ng/ml
LH	<0.0001	1000 ng/ml
TSH	<0.0001	1000 ng/ml

References:

1. Stenman UH, Tanner P, Ranta T, Schroeder and Seppala M. Obstet Gynecol, 1882; 59: 375-377.
2. Kosasa TS. J Reprod Med, 1981; 26:201.
3. Dipietro S. Laboratory Management, 1981; 19: 1.
4. Uotilia M, Ruoslahti E and Engvall I. J Immunol Methods, 1981; 42: 11-15.
5. Masseyeff R and Maiolini R. J Immunol Methods, 1975; 8: 233.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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