

hCG ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the
Quantitative Determination of
hCG Concentration in Human
Serum/Plasma

(For In Vitro Diagnostic Use Only)

Catalogue No. PT-hCG-96

PISHTAZ TEB DIAGNOSTICS

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.

The hCG EIA test provides a rapid, sensitive and reliable assay.

Test Principle

The hCG Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay (Sandwich ELISA). The assay system utilizes one anti-hCG antibody phase (microtiter wells) immobilization and another mouse monoclonal anti-hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwich formation of hCG between solid phase and conjugated antibodies. After second wash step a solution of TMB is added which resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of hCG is directly proportional to the color intensity of the test sample.

Materials provided with kit

1. Antibody coated wells(1 plate, 96 wells): Microtiter wells coated with monoclonal anti hCG.
2. Enzyme conjugate (1 vial, 12 ml): Monoclonal anti HCG labeled with HRP in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
3. Standards set (1 ml /vial): contains 0.0 (2 ml), 25, 100, 250, 500 and 1000 IU/L of hCG WHO 3rd International Standard from NIBSC / code 75 / 537 diluted in buffer containing protein as stabilizer and 0.05% Thiomerosal as preservative.
4. Low control serum (1 ml / vial): Contains certain amount of hCG diluted in buffer containing protein as stabilizer and 0.05% Thiomerosal as preservative.

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5. High control serum (1 ml / vial): Contains certain amount of hCG diluted in buffer containing protein as stabilizer and 0.05% Thiomerosal as preservative.
6. Assay buffer (1 vial, 12 ml): Contains phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use.
7. Chromogen substrate reagent (1 vial, 12 ml): Contains tetramethyl benzidine and hydrogen peroxide, ready to use solution.
8. Wash solution: (1 vial, 50 ml): contains phosphate buffer salt Solution with 0.05 % Tween 20 as detergent, pH = 6, Concentrated (20X).
9. Stop solution (1 vial, 12 ml): Contains hydrochloric acid (1 M) pH<1.
10. Cardboard sealer.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Test Procedure

1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 20 µl of each standard, serum control and specimen in appropriate wells.
3. Add 100 µl of assay buffer into each well and mix gently for 15 seconds.
4. Seal the plate with cardboard sealer and incubate wells for 30 minutes at room temperature (22-28°C).
5. Take out wells contents by flicking the microplate into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution). Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

Materials required but not provided

1. Precision pipettes: 20 and 100 µl
2. Disposable pipette tips.
3. Distilled water.
4. Absorbent paper or paper towel.
5. Graph paper.
6. Microtiter well reader.

General Information

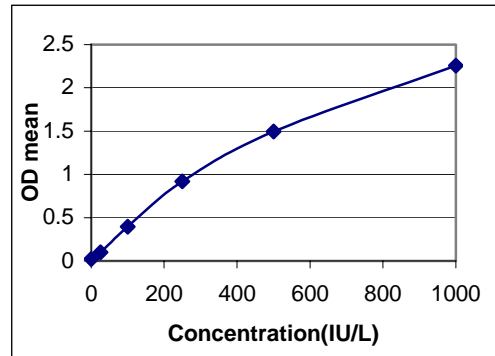
1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8 °C upon receipt and when it is not in use.



6. Add 100 µl of Anti-hCG-HRP conjugate into the wells.
7. Seal the plate with cardboard sealer again. Leave wells for 30 minutes at room temperature (22-28°C).
8. Repeat step 5.
9. Dispense 100 µl of chromogen/ substrate solution into the microplate wells.
10. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
11. Stop the reaction by adding 100 µl of stop solution to the microplate wells.
12. Measure absorbance at 450 nm by ELISA reader. (Use 630 nm filter as reference filter if it's available).



Note: All absorbance shown in above curve are for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curves.

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/L on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample, determine the corresponding concentration of hCG in IU/L from the standard curve.

Expected Values

Each laboratory must establish its own normal ranges based on patient population. hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The normal value for HCG during pregnancy was shown below:

After implantation	Expected value (IU/L)
First month	0-56500
2-3 month	7650-288500
Second trimester	3600-106000
Third trimester	4200-117000

Example of Standard curve

Standards (IU/L)	Absorbance
0	0.021
25	0.101
100	0.395
250	0.921
500	1.495
1000	2.255

During the first 6 weeks after implantation, serum hCG levels doubled each 2 days but after child birth, hCG level fall rapidly and disappear in a short period. Low levels of hCG are reported in ectopic pregnancy and in case of threaten abortion. Elevated hCG levels are frequently associated with trophoblastic and non trophoblastic neoplasmas; these conditions should be considered before a diagnosis of pregnancy can be made.

Interpretation of results:

Suspected: Those serums with **10-25 IU/L** of hCG should be considered as suspicious or suspected.

Positive: Sera which contain more than **25 IU/L** of hCG should be considered as positive.

Negative: Sera which contain less than **10 IU/L** of hCG should be considered as negative.

In the case of negative or suspicious serum, samples should be taken at least after 48 hours and re-tested again.

To achieve reliable and precise results in those sera with high level of hCG (> 1000 IU/L), sera should be diluted with 0 standard and result calculated considering dilution factor.

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of zero standard + 3 SD, the minimum detectable concentration of hCG by this assay is estimated to be 1 IU/L.

2. Test Precision

Three serum samples with different concentrations of hCG were repeatedly tested. Results are shown in table 1 and 2:

Table 1: Intra-assay

No.	No. of Tests Performed	Means IU/L	SD IU/L	CV%
1	24	14.5	0.8	5.5
2	24	228	8.2	3.5
3	24	890	45	5.0

Table 2: Inter-assay

No.	No. of Tests Performed	Means IU/L	SD IU/L	CV%
1	10	15.9	1.12	7.0
2	10	231	10.5	4.6
3	10	906	56.8	6.3

*Each test has been run in duplicate

3. Test Recovery

To assess test recovery, certain amount of hCG was added into 4 different sera with known concentrations of hCG and then sera were tested. The recovery was determined and results are shown below:

Table 3: Test recovery

No.	hCG level IU/L	hCG added IU/L	Exp. value IU/L	Obs. IU/L	Rec (%)
1	25	25	25	24	96
1	25	250	137	144	105
1	25	1000	512	501	98
2	131	25	78	73	93
2	131	250	190	202	106
2	131	1000	565	555	98
3	365	25	195	202	103
3	365	250	307	315	103
3	365	1000	682	690	101
4	840	25	432	425	98
4	840	250	545	565	104
4	840	1000	920	900	98

Exp.: Expected, Obs.: Observed,
Rec.: Recovery

4. Test Linearity

To verify test linearity, 4 different serum samples with known concentration of hCG were serially diluted with 0 standard. Then the serums were tested. The results and serum recovery were determined considering dilution factor:

Table 4: Test linearity

No.	hCG (IU/L) undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	435	94	105	100	96
2	814	95	102	97	99
3	1370	98	100	93	99
4	1890	101	98	96	98

5. Test Specificity

To verify cross reaction of kit with other closely related hormones, the following hormones were tested for cross-reactivity:

Table 5: Specificity test result, Cross reaction

Analyte name	Concentration	Apparent concentration hCG (IU/L)
hFSH (IU/L)	1000	<1
	500	<1
	200	<1
	100	<1
hTSH (mIU/L)	1000	<1
	500	<1
	200	<1
	100	<1
hLH (IU/L)	1000	<1
	500	<1
	200	<1
	100	<1

6. Hook effect




The hCG assay was done on sera with high concentrations of hCG (up to 500,000 IU/L) and no "hook effect" was seen.

References

1. Bagshave, K.D. (1984) Clinical applications of hCG. *Adv. Exp. Med Biol.* 176:313-324.
2. Braunstein, G.D., Rasor, J. and Adler, D. et al (1976) Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am. J. Obst. Gynec.* 126:678.
3. Saller, B., Clara, R. and Spottl, G. et al (1990) Testicular cancer secretes intact human choriogonadotropin (hCG) and its free B-subunit: Evidence that hCG (+hCG-B) assays are the most reliable in diagnosis and follow-up. *Clin. Chem.* 36/2:234-239.
4. Bogart, M.H. Pandian, m.R. and Jones, O.W. (1987) Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. *Prenatal diagnosis* 7:623-630.

hCG Test Procedure




Step 1

Reagent	Standard	Control Serum	Sample
			
Standard	20 μ l	None	None
Control Serum	None	20 μ l	None
Sample	None	None	20 μ l
Assay Buffer	100 μ l	100 μ l	100 μ l

Mix wells gently for 15 seconds and Incubate microplate wells for 30 minutes at room temperature.

Step 2




Tap plate contents into a waste container. Wash the microplate wells for 5 times according to test manual.

Anti hCG-HRP conjugate	100 μ l	100 μ l	100 μ l
			

Incubate microplate wells for 30 minutes at room temperature.





Step 3

Tap plate contents into a waste container. Wash the microplate wells for 5 times according to test manual.

Chromogen-substrate solution	100 μ l	100 μ l	100 μ l
			

Incubate wells for 15 minutes at room temperature and in dark place.


Step 4

Stop Solution	100 μ l	100 μ l	100 μ l
			

Measure absorbance at 450 nm by ELISA reader and calculate the results.