



FREE β hCG - Elisa

For the quantitative determination of free beta subunit of human chorionic gonadotropin (free β -hCG) concentration in human serum



BL-45-E

IN VITRO DIAGNOSTIC USE

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1 INTENDED USE

The free beta hCG quantitative assay is designed for quantitative measurement of chorionic Gonadotropin free beta subunit in human serum.

Not be used for risk calculation of Trisomie 21.

2 CLINICAL APPLICATION

1. Tumour marker test in trophoblastic tumours
Hydatiform moles and choriocarcinomas may secrete large amounts of native hCG and its two free subunits α and β into the peripheral blood circulation.
2. Tumour marker test in non-trophoblastic cancers
3. Diagnostic and monitoring test in pregnancy
hCG and its free subunits α and β appear in the serum and urine of pregnant women about 9 days following ovulation. The Free β hCG level then increases rapidly to reach a peak between the 8th and the 12th week.
Although Free β HCG measurement may be of clinical interest in monitoring of pregnancy when applied to serum, this assay is not intended to be used as a screening parameter in Trisomie 21.

3 INTRODUCTION


Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit.

4 PRINCIPLE OF THE ASSAY

The free β -hCG ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay¹². The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the β -subunit of the hCG molecule. Mouse monoclonal anti- β -hCG antibody is used for solid phase immobilization (on the microtiter wells). A polyclonal rabbit anti- β -hCG antibody is conjugated with horseradish peroxidase. The test sample is allowed to react sequentially with the two antibodies, resulting in the β -hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation two separate 30 minute incubations at 37 °C, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of β -hCG is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

5 REAGENTS

5.1 Materials provided with the kit

1.  **Murine Monoclonal Anti- β -hCG antibodies coated microtiter wells**
96 wells
2.

CAL	N
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Reference Calibrator Set (lyophilized)
N=0 to 5
See exact values on the vial labels
Please see "Reagent Preparation"
3.

DIL	BUF
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Dilution Buffer (Sample Diluent)
13 ml
Ready to use
4.

Ab	HRP
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Enzyme Conjugate Reagent 18 ml
ready to use,
Rabbit anti- β -hCG conjugated to horseradish peroxidase
5.

CHROM	TMB
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TMB Reagent
11 ml
Ready to use
6.

STOP	SOLN
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Stop Solution
11 ml
Ready to use

5.2 Material required but not provided

1. Precision pipettes: 50 µl, 100 µl, 150 µl and 1.0 ml
2. Disposable pipette tips
3. Distilled water
4. Vortex mix or equivalent
5. Absorbent paper or paper towel
6. Graph paper
7. Microtiter plate reader (450 ± 10 nm), optical density range of 0-2 OD or greater

6 SPECIMEN COLLECTION AND HANDLING

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

If sera can not be assayed immediately, they can be stored at 2-8°C or frozen.

7 STORAGE AND STABILITY OF KIT REAGENTS

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.

8 REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. **Calibrators:** Reconstitute each lyophilized calibrator with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted calibrators will be stable for up to 30 days when stored sealed at 2-8°C.

9 PROCEDURAL NOTES

1. It is very important to wash the microwells thoroughly and remove the last droplets of water to get the best results.
2. Pipet all reagents and samples into bottom of the well.
3. Absorbance is the function of the time and temperature of incubations. It is recommended to have all reagents and sample caps removed, all needed wells secured in holder and signed. It will ensure the equal elapsed time for each pipetting without interruption.

10 ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense **50 µl of Calibrators, Controls and Serum Samples** into appropriate wells.
3. Dispense **100 µl of Zero Buffer** into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at **37°C for 30 minutes**.
6. Remove the incubation mixture by decanting or aspirating the contents.
7. Rinse and wash the microtiter wells **5 times** with distilled water or deionized water. (Please do not use tap water). After the last wash strike the wells sharply onto absorbant paper to remove all residual water droplets.
8. Dispense **150 µl of Enzyme Conjugate Reagent** into each well. Gently mix for 5 seconds.
9. Incubate at **37°C for 30 minutes**.
10. Remove the incubation mixture by decanting or aspirating the contents.
11. Rinse and wash the microtiter wells **5 times** with distilled water or deionized water. (Please do not use tap water). After the last wash strike the wells sharply onto absorbant paper to remove all residual water droplets.
12. Dispense **100 µl of TMB solution** into each well. Gently mix for 5 seconds.
13. Incubate at **room temperature for 20 minutes**.
14. Stop the reaction by adding **100 µl of Stop Solution** to each well.
15. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
16. Read optical density at **450 nm** with a microtiter well reader **within 15 minutes**.

11 CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in mIU/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of β -hCG in mIU/ml from the calibrator curve.

12 LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

13 EXAMPLE OF CALIBRATOR CURVE

Results of a typical calibrator run with optical density readings at 450 nm shown in the Y axis against β -hCG concentration shown in the X axis. This calibrator curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and calibrator curve.

β -hCG (ng/ml)	Absorbance (450 nm)
0	0.061
2.5	0.296
5.0	0.498
10.0	0.929
25.0	1.711
50.0	2.613

14 STANDARDIZATION

For intact hCG, 1 ng is approximately equivalent to 15 mIU (WHO, 1st IRP 75/537). For free β -hCG subunit, since there is no WHO standardization, we tested the free β -hCG against hCG's ELISA kit, and found 1 ng of free β -hCG equals to 0.1 mIU in terms of hCG immunological activity.

15 EXPECTED VALUES AND INDICATIONS FOR QUANTITATIVE FREE β -HCG ASSAY

The following information is cited from reference no.12:

1. hCG and Free β -hCG Subunit Levels in Normal Pregnancy

A logarithmic increase in the serum concentration of hCG was observed from 5-8 weeks of gestation (2,600 ng/ml to 33,000 ng/ml) as defined by last menstrual period; thereafter, hCG values decreased. Similarly, free β -hCG levels increased rapidly to reach maximum levels (~60 ng/ml) at 8-9 weeks of pregnancy, followed by a gradual decline during the next 11-12 weeks of gestation. At 5 weeks of gestation, the ratio of free β -hCG to intact hCG is approximately 1.0 % (w/w). Thereafter, this ratio remains remarkably constant over 22 weeks of gestation (~ 0.5 % w/w).

2. hCG and Free Subunits Levels in Gestational Choriocarcinoma

Free α and free β -subunits and hCG levels were measured in five patients with untreated gestational choriocarcinoma. The concentrations in serum are shown in the following table.

Patient Number	hCG (ng/ml)	Free α -hCG (ng/ml)	Free β -hCG (ng/ml)
1	210,000	112	8,000
2	22,195	20	1,300
3	6,840	1	232
4	36,000	44	3,900
5	4,200	2	350

The levels of free α -hCG were low, ranging from 1-112 ng/ml, whereas hCG levels ranged from 4,200 to 210,000 ng/ml (1 ng \approx 15 mIU). In contrast, free β -hCG concentrations were found to be markedly elevated in choriocarcinoma.

3. Ectopic Production of hCG and Free Subunits by Nontrophoblastic Tumors

The following table shows results obtained in various tumors and healthy and benign disease controls:

Measurement of hCG, α -hCG, and β -hCG serum levels in nontrophoblastic tumors, benign disease, and healthy controls

Tumor type	No. of samples	hCG (ng/ml)	α -hCG (ng/ml)	β -hCG (ng/ml)
Cervix	20	0	1 (1.6) ^a	1 (0.65)
Corpus uterus	20	0	0	0
Gastric	20	0	0	1 (1.5)
Pancreatic	20	0	1 (16.0)	2 (0.8, 3.1)
Colon	20	0	0	0
Lung	20	0	1 (90.0)	1 (0.7)
Ovarian	20	0	1 (1.8)	0
Prostate	20	0	1 (1.6)	0
Other digestive tract tumor	18	0	0	0
Total [%]	178	0	5 [3]	5 [3]
Benign disease controls	61	0	1 (1.6)	0
Healthy controls	50	0	0	0
Total [%]	11	0	1 [1]	0

^a The number in parentheses represents the measured value in ng/ml. The cut-off values for positive results are 1.5 ng/ml for hCG and α -hCG and 0.4 ng/ml for β -hCG.

When compared with healthy control values, all nontrophoblastic cancer patients had hCG concentration within the normal range (~ 0.9 ng/ml). Free subunits were elevated in 10 of 178 patients. It is noteworthy that α -hCG levels in two patients (pancreatic and lung tumors) were relatively high (16 and 90 ng/ml, respectively), whereas the maximum concentration of free β -hCG was only 3.1 ng/ml (pancreatic tumor).

16 PERFORMANCE CHARACTERISTICS

16.1 Specificity

Cross-reactivity

Antigens	Conc.	Equivalent hCG	% Cross-Reactivity
TSH	1,000 ng/ml	0.0 ng/ml	0.00
FSH	5,000 ng/ml	0.0 ng/ml	0.00
Prolactin	1,000 ng/ml	0.68 ng/ml	0.07
LH	1,000 ng/ml	4.88 ng/ml	0.49
A-hCG	5,000 ng/ml	1.25 ng/ml	0.03
Intact hCG	1,000 ng/ml	7.24 ng/ml	0.72

16.2 Sensitivity

The minimal detectable concentration of β -hCG in this assay is estimated to be 0.25 ng/ml.

17 REFERENCES

- Engall, E., Methods in Enzymology, volume 70, Van Vunakis, H. and Langone, J.J. (eds.), Academic Press, new York, 419-492 (1980).
- Uotila M., Ripsöajto, E. and Engvall E., J.Immunol. Methods, 42, 11-15 (1981)
- Brizot, ML, Jauniaux E. Mckie AT, Farzaneh F and Niclaides KH. Hum. Reprod. 1995; 10; 2506-9.
- Forest JC, Masse J, Rousseau F, Moutquin JM, Brideau NA and Belanger M. Clin. Biochem. 1995; 28:443-9.
- Breimer L. ann.Clin.Biochem 1995:32:233.
- Loncar K, Barnabei VM, and Larsen JW Jr. Obstet. Gynecol. Surv. 1995; 50:316-20
- Densem J., and Wald NJ. Prenat. Diagn, 1995; 15:94-5.
- Ozturk M, Berkowitz R, Goldstein D, Bellet D, Wands JR. Am J Obstet Gynecol 1988; 158:193-8.
- Wald NJ, Cuckle HS, Densem JW, et al. Br Med J 1988; 297:883-7.
- Hay DL. BR J Obstet Gynaecol 1988; 95:1268-75.
- Macri JN. et al. Am J Obstet Gynecol 1990; 163:1248-53.
- Ozturk M, et al. Endocrinology 1987; 120:499-508.
- Cole LA. et al. Endocrinology 1983; 113: 1176
- Gaspard UJ et al. Clin Endocrinol (OXF) 1980; 13:319.