



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com



See external label



2°C-8°C



Σ=96 tests



Cat # 4221Z

FREE BETA-SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN (Free β-hCG)

Free Beta HCG

Cat # 4221Z

Test	Free Beta HCG
Method	Enzyme Linked Immunosorbent ELISA
Principle	Peroxidase – Conjugated ELISA
Detection Range	0-50mIU/mL
Sample	20µl serum
Specificity	100%
Sensitivity	0.5mIU/ml
Total Time	~ 80 min
Shelf Life	12 -14 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

Intended use

The free beta hCG-subunit quantitative assay is designed for in vitro quantitative measurement of human chorionic gonadotropin free beta-subunit in patient's serum. (For professional use only).

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. In the normal second-trimester maternal sera, the level of intact hCG range from 20,000 mIU/ml to 50,000 mIU/ml. In contrast, the levels of either free α - or free β -hCG are on average one half of 1% of hCG levels. hCG and the free subunits appear not to be useful as serological markers for nontrophoblastic tumors; however, the absolute increase of β -hCG level in choriocarcinoma patients clearly differentiates it from normal pregnancy.

Recent studies showed a significant increase in the level of free β -hCG subunit in trisomy 21 cases as compared with controls. Hence, it has been suggested that free β -hCG subunit assay in a combination of maternal serum AFP could be effective in a screening protocol for trisomy 21.

Principle of the test

The free β -hCG Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti- β -hCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti- β -hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the β -hCG antibody coated microtiterwells and incubated with the Zero Buffer. If β -hCG is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and β -hCG antibody labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the β -hCG on the well, resulting in the β -hCG molecules being sandwiched between the solid phase and enzyme- linked antibodies. After an incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of β -hCG is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

1. Antibody-coated microtiter wells.
2. Reference standards, 0, 2.5, 5, 10, 25, 50 mIU/ml, in the sample diluent against WHO IRP 75/551. (1 mIU/ml = 1 ng/ml for β -hCG). Lyophilized standards, reconstitute with 0.5 ml distilled water before use.
3. Zero Buffer (Sample diluent), 20 ml
4. Enzyme Conjugate Reagent, 18 ml
5. TMB Substrate, 12 ml.
6. Wash Buffer Concentrate (50X), 15ml.
7. Stop Solution, 12 ml.

Materials required but not provided:

1. Precision pipettes: 0.04~ 0.2ml, 0.2~1.0ml
2. Disposable pipette tips
3. Distilled water
4. Vortex mixer or equivalent
5. Absorbent paper or paper towel

6. Graph paper
7. Microtiter well reader

Specimen collection and preparation

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.

Storage of test kits and instrumentation

1. 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. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

1. All reagent should be brought to room temperature (18-22°C) before use.
2. Reconstitute each lyophilized standard with **0.5** ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and it will be stable for at least two weeks at that conditions.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer Concentrate(50x) into distilled water to prepare 750 ml of washing buffer(1x). Mix well before use.

Assay procedures

1. Secure the desired number of coated wells in the holder.
2. Dispense **50**µl of standard, specimens, and controls into appropriate wells.
3. Dispense **100**µl of Zero Buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have completed mixing in this setup.
5. Incubate at 37°C for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a sink.
7. Rinse and empty the microtiter plate 4 times with washing buffer(1X) and 1 time with distilled water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at 37°C for 30 minutes. Remove the incubation mixture by flicking plate contents into sink.
11. Rinse and empty the microtiter plate 4 times with washing buffer(1X) and 1 time with distilled water.
12. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
13. Dispense 100µl TMB solution into each well. Gently mix for 5 seconds.
14. Incubate at room temperature in the dark for 20 minutes.
15. Stop the reaction by adding **100**µl of Stop Solution to each well.
16. Gently mix for 5~30 seconds. *It is very important to make sure that the blue color changes to yellow color completely.*
17. Read optical density at 450nm with a microtiter plate reader within 30 minutes.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance reading.

Limitations and Precaution:

1. The free beta hCG-subunit quantitative assay is designed for in vitro use only. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
2. The absorbance of this quantitative assay is to 50 mIU/ml of β -hCG. It is recommended that samples falling above the range should be diluted with sample diluent (Zero Buffer) to absorbance that within the standard curve range.

Calculation of results

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

Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y axis against hCG concentrations shown in the X axis. This standard 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 standard curve.

β -hCG (mIU/ml)	Absorbance (450nm)
0	0.021
2.5	0.122
5.0	0.247
10.0	0.573
25.0	1.662
50.0	3.231

Expected Values and Indications for Quantitative Free β -hCG Assay:

1. In early pregnancy, free β -hCG concentration was found 10~80 mIU/ml. The free β -hCG /intact hCG ratio was found 3.08~3.28 percents. After 6 to 7 weeks the free β -hCG and the ratio value declined. During the second and third trimester, a constant ratio was observed about 1 percent.
2. Serum samples from 40 normal subjects were assayed, in this population, 99% of the values were less than 0.4 mIU/ml. Serum hCG and free subunit levels in sera from patients with gestational choriocarcinoma were reported as following: (Ozuturk et al. Endocrinology, 1987)

Patient No	HCG (mIU/ml)	α -hCG (mIU/ml)	β -hCG(mIU/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

Performance Characteristics

1. Precision

1]. Intra-Assay:

	Replicates	Mean	S.D.	% CV
Level I	16	4.22	0.09	2.23
Level II	16	8.54	0.21	2.49
Level III	16	20.16	1.23	6.09

2]. Inter-Assay:

	Replicates	Mean	S.D.	% CV
Level I	16	4.36	0.29	6.70
Level II	16	9.22	0.65	7.10
Level III	16	20.05	1.42	7.10

2. Linearity

Two patient sera were serially diluted with Sample Diluent in a linearity study. The average recovery was 103.4 %.

Sample A			
Dilution	Expected	Reading	% Rec.
Undiluted	26.1	26.1	
x2	13.1	12.9	98.7
x4	6.5	5.9	90.8
x8	3.3	3.1	93.6
x16	1.6	1.8	113.0
Avg.:		99.0	
Sample B			
Dilution	Expected	Reading	% Rec.
Undiluted	36.6	36.6	
x2	18.3	21.3	116.3
x4	9.2	9.3	101.8
x8	4.6	4.4	96.0
x16	2.3	2.4	103.4
Avg.:			104.4

3. Recovery

Various patient serum samples of known ?-hCG levels were mixed and assayed in duplicate. The average recovery was 99.95 %.

Expected Concentration	Observed Concentration	% Recovery
37.47	35.59	95.0
21.40	19.99	93.4
11.36	11.68	102.8
6.07	5.90	102.9
3.04	2.98	102.0
2.02	1.95	103.6

Average Recovery: 94.9 %

4. Sensitivity

The minimum detectable concentration of this assay is estimated to be 0.5 mIU/mL.

5. Cross-reactivity

The following human materials were tested for cross reactivity of the assay:

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

6. Hook Effect

No hook effect was observed in this assay.

References

1. Brizot ML, Jauniaux E, Mckie AT, Farzaneh F, and Nicolaidis KH. Hum Reprod 1995; 10: 2506-9
2. Forest JC, Masse J, Rousseau F, Moutquin JM, Brideau NA, and Belanger M. Clin Biochem 1995; 28: 443-9
3. Breimer L. Ann Clin Biochem 1995; 32: 233
4. Loncar K, Barnabei VM, and Larsen JW Jr. Obstet Gynecol Surv 1995; 50: 316-20
5. Densem J, and Wald NJ. Prenat Diagn 1995; 15: 94-5
6. Ozturk M, Berkowitz R, Goldstein D, Bellet D, Wands JR. Am J Obstet Gynecol 1988; 158:193-8
7. Wald NJ, Cuckle HS, Densem JW, et al. Br. Med J 1988; 297:883-7
8. Hay DL. Br. J Obstet Gynaecol 1988; 95:1268-75
9. Macri JN, et al. Am J Obstet Gynecol 1990; 163:1248-53
10. Ozturk M et al. Endocrinology 1987; 120:499-508
11. Cole LA, et al. Endocrinology 1983; 113:1176
12. Gaspard UJ et al. Clin Endocrinol (OXF) 1980;13:319

Date Adopted	Reference No.
2008-05-01	DA-Free B-hCG-2009



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003



Revision Date: 10/10/2009