Instructions for Use

Total β-Human Chorionic Gonadotropin (CLIA)

[Product Name]

Total β-Human Chorionic Gonadotropin (CLIA)

[Packing Size]

24×1 Tests/Pkg (Calibrators included);

60×1 Tests/Pkg (Calibrators included);

60×1 Tests/Pkg

[Intended Use]

Total β-HCG assay is a chemiluminescent immunoassay (CLIA) for quantitative measurement of the total β subunit of human chorionic gonadotropin (Total β-HCG) in human serum, plasma, and whole blood samples. It is mainly used for auxiliary diagnosis of ectopic pregnancy and early pregnancy.

Human chorionic gonadotropin (HCG) is a member of the glycoprotein hormone family, and mainly secreted by trophoblast cells of placenta. It has a molecular weight of 45~50kDa [1]. It is composed of heterodimers of 237 amino acids, which are connected by non-covalent bonds of α and β subunits through charge interaction, and contains a total of eight carbohydrate side chains [2, 3]. The α subunit of HCG is similar to luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid stimulating hormone (TSH) of other glycoprotein hormone families [3, 4], while the structure of β subunit is different. Although HCG β subunit and LH β subunit have 80-85% homology, but HCG has 24 more amino acids than LH β-subunit [3, 4]. Thus, the β subunit determines the biological and immunological properties of HCG. It mainly maintains the growth of luteum, promotes the aromatization of androgen into estrogen, and stimulates the formation of progesterone, thus providing protection for the endometrium [5, 6]. The hormone is secreted by trophoblastic cells 9-13 days after implantation of the fertilized egg in the endometrium [7]. After conception, HCG levels in blood rise rapidly and reaches the peak around the 8th week of gestation, and then decline slowly until the 18th -20th week after gestation. After baby delivery, HCG concentration returns to the normal levels as in non-pregnant women. Therefore, total β-HCG is a reliable marker for early pregnancy diagnosis. In addition, highly sensitive and quantitative measurement of total β -HCG level in blood can predict threatened abortion [3, 8], and assist in the detection of abnormal pregnancy [9, 10] and multiple pregnancy [11, 12].

[Principle of the Assay]

This reagent adopts the double-antibody sandwich chemiluminescent immunoassay format. The assay principle is described below:

(1) Sample, anti-β-HCG antibody coated paramagnetic microparticle, and reaction diluent are combined and incubated. β-HCG in the sample will bind to anti-β-HCG antibody coated microparticle, and then, a magnet is used to capture the microparticle, and the mixture will be washed to remove unbound substance.

(2) Another anti-β-HCG antibody labeled with acridinium ester is added to the reaction well. After mixing and incubation, the acridinium labeled anti-β-HCG antibody binds to another site of β-HCG in the sample to form an antigen-antibody sandwich complex.

(3) After the reaction is completed, a magnet captures the microparticle, and then unbound substance is washed off. Add pre-trigger and trigger solution to the reaction mixture sequentially to initiate chemiluminescence reaction.

(4) A photomultiplier tube is used to measure photons generated from the reaction. The count is proportional to the quantity of β-HCG in the sample. Concentration of β-HCG is determined by an internal calibration curve.

[Main Components]

Packing Size

Component Name \ Packing Size	Fill Volume		
	24×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg
Total β-HCG Reagent Cartridge – Microparticle (R1)	24×50 μL	60×50 μL	60×50 μL
Total β-HCG Reagent Cartridge – Conjugate (R2)	24×100 μL	60×100 μL	60×100 μL
Total β-HCG Reagent Cartridge – Reaction diluent (R3)	24×150 μL	60×150 μL	60×150 μL
Total β-HCG Calibrator – Total β-HCG Calibrator C1	1×1.0 mL	1×1.0 mL	/
Total β-HCG Calibrator – Total β-HCG Calibrator C2	1×1.0 mL	1×1.0 mL	/
Calibration Card – Calibration curve and information about calibrators	1 pcs	1 pcs	/

Main Composition

Component		Main Composition
Total β-HCG Reagent Cartridge	Microparticle (R1)	Mouse anti-β-HCG antibody coated microparticle, ~0.3 g/L; Tris buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Conjugate (R2)	Acridinium labeled mouse anti-β-HCG antibody, ~10 μg/L; MES buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Reaction diluent (R3)	Tris buffer, 50 mmol/L; ProClin 300, 0.5g/L
Total β-HCG Calibrator C1		Total β-HCG (Human); HEPES buffer, 50 mmol/L; ProClin 300, 0.5 g/L
Total β-HCG Calibrator C2		Total β-HCG (Human); HEPES buffer, 50 mmol/L; ProClin 300, 0.5 g/L
Calibration Card		Calibration curve and information about calibrators

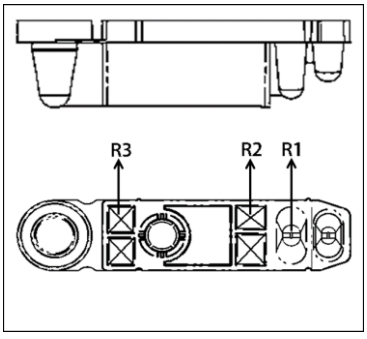
Note:

(1) Components in different lots of reagent cannot be mixed or exchanged for use.

(2) Traceability: This quantification method can be traced back to the National Standard for human chorionic gonadotropin, No. 150555.

(3) Information about calibrators can be found on the screen interface of the instrument after scanning the Calibrator Card (such as lot number and concentration etc.)

The position of each component is shown in the front view (Upper) and vertical views (Down) of the reagent pack.



Instruments and accessories needed but not supplied (available from Medcaptain)

(1) Medcaptain Immu F6 automatic chemiluminescent immunoassay analyzers; Medcaptain Immu F6S automatic chemiluminescent immunoassay analyzers;

(2) Pre-trigger solution;

(3) Trigger solution;

(4) Washing solution;

(5) 500μL pipette tips;

(6) Sexual Hormone Controls;

(7) Sample diluent.

[Storage and Shelf-life]

Storage: Store sealed reagent cartridges and calibrators at 2～8℃ in upright position, and avoid freezing

Shelf Life: 14 months.

Stability of Calibrators: Sealed vial of calibrators can be kept at 2~8℃ in dark for 14 months. After calibrator C1 and C2 is uncapped, it can be stored for 5 day at 10~30℃, and for 60 days at 2~8℃.

The dates of manufacturing and expiration can be found on the labels.

[Matched Instruments]

Medcaptain Immu F6 Automatic Chemiluminescent Immunoassay Analyzer; Medcaptain Immu F6S Automatic chemiluminescent Immunoassay Analyzers

[Specimen Types]

Serum, plasma, and whole blood sample (collected with EDTA-K_2, EDTA-K_3, lithium heparin, and sodium heparin as anti-coagulants) can be used in the testing.

Sample volume for each test: 10 μL.

The collected sample should be tested as soon as possible.

Whole blood can be kept at 10~30℃ for 4 hours, and it is recommended to complete the sample testing within 4 hours after sample collection.

Serum and plasma can be kept at 10~30℃ for 24 hours, at 2～8℃ for 7 days, and at -20℃ or below for 12 months. Frequent freeze-thaw cycle should be avoided, and only one freeze-thaw cycle is allowed. If the sample contains precipitate or frozen floccule, centrifugation is needed to clear the sample before testing.

Sample collection tubes from different manufacturers may lead to variation in testing results, due to difference in tube material and additives in the tubes. Medcaptain has not evaluated all types of sample collection tubes from different manufacturers. Each laboratory should make its own judgment about the usability of sample collection tubes.

[Testing Procedure]

Reagent Preparation

Reagent: Total β-HCG reagent cartridge (containing magnetic microparticle R1, acridinium labeled conjugate R2, and reaction diluent R3) is ready for use. It can be loaded directly into instrument after opening the package.

Calibrators: Calibrator C1 and C2 are ready for use. It can be added to sample tubes separately, and loaded directly to the sample rack for testing.

Calibration

Refer to the relevant chapters in the operation manual of each chemiluminescent immunoassay analyzer.

Calibration tests should be ordered before the first time use of Total β-HCG assay. Medcaptain provides Total β-HCG reagent pack and matched calibrators to calibrate the instrument.

Before calibration, scan the calibration card provided in the reagent kit, the built-in calibration curve and calibrator information will be scanned into the system.

Before calibration testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put calibrators on a sample rack, load calibrators into the instrument.

On the screen interface of “Reagent > Request Calibration”, select test name and lot number to request a calibration.

Select the position of each calibrator on the sample rack, set the number of repeat tests, start calibration.

Automatic immunoassay analyzer makes use of calibration test data to validate the calibration curve, and adjust the calibration curve automatically.

Instrument calibration is effective for 60 days.

A new calibration is needed in the following situation:

(1) Change into a new lot of reagent;

(2) Control test results are out of the target range;

(3) The lot of reagent has been used on the same instrument for more than 60 days.

Refer to the Chapter of “Calibration” in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about calibration.

Control Testing

Sexual Hormone Controls are matched with total β-HCG reagent. There are two levels of controls: Low Control (L) and High Control (H).

These two-level controls should be tested in accordance with any local applicable regulations. Control testing is highly recommended every time the lot of reagent has been changed, the instrument has been re-calibrated, or after trouble shooting/ maintenance service.

Before control testing, take out reagent cartridges from the package, and load all of them into the instrument. The instrument scans the two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put controls on a sample rack, and load it into the instrument;

Select “Control” on the interface of test menu, select test name and control lot;

Click on “Start” and begin the testing. Check the results after control test is finished.

Control testing results should fall into a specific range. If it is out of the target range, the user should check the system, such as expiration date of the controls, storage condition, instrument performance and status. After root cause analysis and correction, the user should test controls again. If the same problem exists, please contact customer service of Medcaptain.

Each laboratory should set up its own control range and frequency of control testing, based on its own practice.

Refer to the Chapter of “Control Testing” in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about control testing.

Sample Testing

Before sample testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of reagent (reagent name, Lot No., and expiration date etc.);

If a sample collection tube is directly loaded to the instrument for testing, the sample volume should be at least 1.0mL;

Uncap sample collection tubes, put samples on a sample rack, and push it into the machine;

Select “Sample” on the interface of test menu, enter information of samples, select test name;

Click on “Start” and begin the testing. Check the results after sample test is finished.

The reagent usage for each test is: R1 50μL, R2 100 μL, R3 130 μL. The instrument aspirates and mixes each component of the reagent cartridge, and incubate at 37℃. Time duration from sampling to result is about 15 min.

Refer to the Chapter of “Sample Testing” in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about sample testing.

Result Calculation

Based on the built-in calibration curve, the instrument automatically calculate the analyte concentration of each sample, in a unit of mIU/mL.

[**Reference Intervals**]

Samples for the study of reference intervals come from local area in Guangdong Province. A total of 123 healthy non-pregnant, premenopausal women without trophoblast disease have been recruited. Serum tests give a reference interval of 95% population at 1.03 mIU/mL. A total of 128 healthy and postmenopausal women have been recruited, serum tests give a reference interval of 95% population at 7.11 mIU/mL. A total of 262 healthy and normal men have been recruited, serum tests give a reference interval of 95% population at 2.02 mIU/mL. If the Total β-HCG test result is between 5 mIU/mL and 25 mIU/mL, it indicates that the individual may be in early pregnancy and the result should be determined according to the overall clinical manifestations of the patient [13-14].

During pregnancy (weeks of pregnancy - defined as completed weeks of pregnancy beginning with the start of the last menstruation phase), the following values have been determined:

Weeks of gestation	N	Total β-HCG (mIU/mL)	
		Median	5^{th}-95^{th} percentile
3	15	17.58	5.82-70.25
4	28	140.57	9.85-760.35
5	26	1397.96	207.88-7135.89
6	22	3340.26	157.84-31788
7	36	39897	3670-162368
8	30	89999	32088-149897

9	28	106266	63785-152135
10	32	85256	46518-186987
12	25	66675	27844-210635
14	26	34465	14056-62035
15	32	28935	12056-69986
16	29	23934	9098.35-56498
17	20	20792	8156.75-55889
18	17	19817	8102.15-58035

Due to the differences in geography, race, sex, and age of tested population, the reference interval may vary in different laboratories. It is highly recommended for each clinical lab to establish its own reference intervals.

[Interpretation of Test Results]

The test data is for clinical reference only. It cannot be used as the only confirmatory evidence, nor to eliminate the possibility of diseases. Clinical diagnosis of patients should take clinical symptoms, body sign, disease history, other lab test results, and treatment response into comprehensive consideration.

Unit conversion formula: Concentration (mIU/mL) ×1= concentration (IU/L).

The measurement range of this assay is: 0.2～10000 mIU/mL. If Total β-HCG concentration is lower than LoD, it will be reported as <0.2 mIU/mL; If Total β-HCG concentration is over the upper limit, it will be reported as >10000 mIU/mL.

For samples with Total β-HCG concentration of >10000 mIU/mL, sample diluent can be used to dilute the sample manually to obtain more accurate result (a dilution factor of 1:100 is recommended). Repeat the test.

When the instrument shows a warning sign of "SMPL", it means insufficient sample volume. Make sure enough sample is added for repeating the test. When the instrument shows a warning sign of "SMPJ", it means the sample probe has been blocked. Clean sample clot in the probe before repeating the test.

Some results are tagged with signs. Refer to the Chapter of "Result Signs" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about results tagged with signs.

[Limitation of the Test Method]

The test data is for clinical reference only. It cannot be used individually as the evidence to confirm or eliminate the possibility of diseases.

There is no Hook effect for samples with Total β-HCG concentration of ≤750,000 mIU/mL.

For endogenous interference substances with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

Endogenous Interference Substance	**Concentration of Interference Substance**
Total Protein	≤12 g/dL
Bilirubin	≤75 mg/dL
Hemoglobin	≤1000 mg/dL
Triglyceride	≤1500 mg/dL

For cross reacting interference substances with concentration less than the value shown in the table below, the measured result of Total β-HCG is less than 1.0 mIU/mL.

Cross reactant	**Concentration**
Follicle-Stimulating Hormone (FSH)	≤500 mIU/mL
Luteinizing Hormone (LH)	≤500 mIU/mL
Thyroid Stimulating Hormone (TSH)	≤100 mIU/L
Human Growth Hormone (HGH)	≤500 ng/mL
HCG-α subunit	≤4000 mIU/mL

For drug as an interference substance with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

Drugs	**Concentration**
Acetaminophen	≤20 mg/dL
Acetylsalicylic Acid	≤20 mg/dL
Ascorbic Acid	≤20 mg/dL
Atropine	≤20 mg/dL
Caffeine	≤20 mg/dL
Ethylenediamine Tetraacetate	≤20 mg/dL
Ethyl Alcohol	≤1 % (V/V)
Gentisic Acid	≤20 mg/dL
Glucose	≤2 g/dL
Salicylic Acid	≤20 mg/dL
Multivitamin	≤0.9 % (V/V)
Ibuprofen	≤50 mg/dL
Heparin (low molecular weight)	≤7200 U/dL

Heterophilic antibodies in human serum may react with immunoglobulin in the reagent or sample, and interfere with immunoassay in vitro. More clinical or diagnostic information is needed to confirm disease status of the patients.

Some patients have frequent contact with animals, or have been treated or diagnosed with mouse monoclonal antibodies. They may have generated heterophilic antibodies. For example, some patients under monoclonal antibody treatment may have human anti-mouse antibodies (HAMA) in blood circulation, leading to false positive or false negative results. Anti-interference components are added to this reagent formulation to minimize the impact of HAMA and ANA, but the problem may not be totally eliminated, and some sample testing may still be impacted. More clinical and diagnostic information is needed to make a solid conclusion.

Samples with the titer of no less than 1:1000 by anti-nuclear IgG test kit (indirect immune-fluorescence method) were studied in the interference tests. It has shown less than ±10% error in the test results.

For RF at a concentration of less than 1500 IU/mL, and for HAMA at a concentration of less than 120 ng/mL, the impact on measurement value is minimal, and the measurement error is within ±10 %.

[Property and Performance]

1. Limit of Blank

LoB ≤ 0.1 mIU/mL.

2. Limit of Detection

LoD ≤ 0.2 mIU/mL.

3. Accuracy

Accuracy should meet at least one of the following criteria:

a) Test the National Standard for human chorionic gonadotropin within the linear range specified by the kit. The relative deviation between the measurement result and the theoretical concentration traced to the National Standard must not exceed 10.0 %.

b) Test the accuracy reference samples at two concentration levels multiple times respectively. The relative deviation between measurement result and t the target value must not exceed 10.0 %.

c) Spike Total β-HCG of a known concentration into real samples at different β-HCG levels. Spiked recovery should be 100±15 %.

4. Linearity

Test Total β-HCG samples with concentration in the range of 1.0～10000 mIU/mL, the linearity correlation coefficient r ≥ 0.9900.

5. Repeatability

Coefficient of variation (CV) for the test results of low (25±5) mIU/mL and high (200±40) mIU/mL corporate reference sample is less than 8.0 %.

6. Lot-to-lot Variation

Coefficient of variation (CV) for the test results of low (25±5) mIU/mL and high (200±40) mIU/mL corporate reference sample with three batches of reagent is less than 10.0 %.

7. Accuracy of Calibrator Value Assignment

Use primary calibrators with assigned values from higher level measurement procedure, calibrate the immunoassay analyzer, and use the same lot of reagent to measure the value of each product calibrator. The measured value of Calibrator C1

and C2 has a relative deviation within ±10.0 % from its assigned value.

8. Homogeneity of Calibrators

8.1 Within-vial Homogeneity

Within-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and CV ≤ 8.0 %.

8.2 Between-vial Homogeneity

Between-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and CV ≤ 5.0 %.

[Attention Notes]

1. It is for in-vitro diagnosis only.
2. It can only be used by professionals.
3. Never use expired reagent kit.
4. Never mix components from different kits, or from different reagent lots.
5. Do not put the reagent cartridges upside down.
6. Measurement of Total β-HCG in a sample using different measurement systems may yield different results, due to the difference in test methods, assay specificity, and factors of interference. The measured values from different systems should not be directly compared to avoid inappropriate clinical interpretation.
7. Strictly follow the protocol in the package insert, and operate according to the lab guidelines.
8. The test results can only be used for clinical reference. Clinical diagnosis of patients should take symptoms, body sign, disease history, other laboratory test results, and response to treatment for comprehensive assessment.
9. User should wear gloves and lab coat. Rinse with water if the skin is in contact with reagent. Flush eyes with copious of water if eyes are in touch with reagent, and see doctor immediately.
10. Take all samples and reaction waste as potential biohazards. All waste must be handled following the local government regulation.
11. This product is a single-use cartridge. Reagent cartridge should be put back into refrigerator and stored at 2~8℃ if they are not opened within the same day.

[Interpretation of Signs]

	Temperature limit.		Date of manufacturing
	*In vitro* diagnostic medical device		Catalogue number
	Batch Code		Consult instruction for use
	Use-by Date		Authorized representative in the European Community
	This way up		CE marking of conformity
	Manufacturer		Unique device identifier
	Biological risks		

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[Basic Information]

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