Lifotronic

CE

Instruction for Use

【Product Name】

Total Beta Human Chorionic Gonadotropin (Total β-HCG) eCLIA

【Package】

REF NO.	Package Size
697039	50T
697040	2×50T
697041	100T
697042	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of human chorionic gonadotropin in human serum and plasma. This assay is intended for use specially in assisting diagnosis and monitoring of pregnancy .

Human chorionic gonadotropin (HCG) is a kind of glycoprotein secreted by placental trophoblastic cells. HCG is composed of two subunits, alpha and beta, containing 237 amino acids, relative molecular mass 37 KD[1,2]. HCG and luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) have similar α- but different β- subunit. HCG α- subunit gene locates on chromosome 6 as the same as TSH, LH and FSH; β-subunit gene locates on chromosome 19, of which difference with TSH, LH and FSH only lies in the last 30 amino acid residues of β-subunits. A last 24 amino acids extension in β-HCG does not exist in β-LH. Therefore, this characteristics of β-subunit is used in a specific assay for HCG to avoid interference of β-LH.

HCG is mainly derived from the trophoblast of placenta syncytium. In addition, the embryo trophoblast, hypophysis of male and unfertilized female also secrete a small amount of HCG.

HCG has ability to maintain menstrual luteal lifecycle; promote androgen conversion to estrogen; inhibit the stimulating effect of plant hemagglutinin on lymphocytes; stimulate fetal testis to secrete testosterone. It can bind to the TSH receptor of maternal thyroid cells and stimulate the active physiological function of thyroid. HCG is began to secrete on the 6th day after fertilization, and can be detected in the serum and urine of pregnant women on the 7th day of gestation, thus can be used for the diagnosis of early pregnancy. Serum concentration peaks at 8 to 10 weeks of gestation, about 50~100 kU/L, and rapidly decreases after 10 days. The blood concentration during middle and late gestation is only 10% of the peak, lasting until childbirth, and generally disappears within 1~2 weeks after birth. HCG in the blood is an important indicator for the diagnosis of ectopic pregnancy, and also the main marker for the diagnosis of trophoblast diseases such as early pregnancy and grapevine pregnancy[5,6].

【Test Principle】

Sandwich principle.Total duration of assay: 18 minutes.

- 1st incubation: a sample, biotinylated monoclonal β-HCG-specific antibody and monoclonal β-HCG-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin.
- Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via calibration and a master curve provided via the reagent barcode.

【Main Components】

The reagent pack consists of MB, RA, RB, sample diluent, calibrators and control materials, different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles: 0.1 M PBS; preservative	1×2.0 mL	2×2.0 mL	1×4.0 mL	2×4.0 mL
(RA)	Anti-β-HCG-Ab~Ru(bpy)$_3^{2+}$: 0.1 M Phosphate buffer; preservative	1×4.0 mL	2×4.0 mL	1×8.0 mL	2×8.0 mL
(RB)	Anti-β-HCG-Ab~biotin: 0.1 M phosphate buffer; preservative.	1×3.5 mL	2×3.5 mL	1×7.0 mL	2×7.0 mL
Sample Diluent	0.1M phosphate buffer, BSA, preservative	1×10.0 mL	2×10.0 mL	1×10.0 mL	2×10.0 mL
Calibrator (High)	0.1M phosphate buffe, preservative	1×1.0 mL	2×1.0 mL	1×1.0 mL	1×1.0 mL
Calibrator (Low)	0.1M phosphate buffe, preservative	1×1.0 mL	2×1.0 mL	1×1.0 mL	1×1.0 mL
Control Material (High)	0.1M phosphate buffe, preservative	1×1.0 mL	2×1.0 mL	1×1.0 mL	1×1.0 mL
Control Material (Low)	0.1M phosphate buffe, preservative	1×1.0 mL	2×1.0 mL	1×1.0 mL	1×1.0 mL

The assignment of calibrators value complies strictly with ISO 17511:2003, and this method can be traced back to human chorionic gonadotropin 07/364 (NIBSC).

See the quality control card for the target value and permissible range of quality control.

Materials and instruments required but not provided:

- Auffer
- Buffer
- Concentrated Washing Buffer
- Full Automated ECL Analyzer
- Assay Cup

【Storage and Shelf Life】

Unopened reagent rackpack, calibrators and control materials should be placed at 2~8ºC and will be valid for 12 months.

Opened reagents, calibrators and control materials should be used and stored at 2~8ºC in 28 days, otherwise trashed.

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Full Automated ECL Analyzer: eCL8000.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, heparin sodium and EDTA-2K anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not in suggestion. Samples would better to be tested in 8 hours after collection, otherwise, should be stored 2~8ºC for no more than 7 days or -25~-15℃, 12 months. Freezing and thawing cycle is permitted only once.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Total β-HCG test according to the operational manual.

Put the Total β-HCG rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

The sample volume required for each HCG test is 5 μL.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card (refer to instrument operational manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) the reagents of different lot are used;

(2) the same lot of reagents were used on the analyzer beyond 28 days;

(3) quality control misses the target.

Quality control

In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. After each calibration, reagent lot change, maintenance or failure repairment, quality control is recommended. The quality control results should fall within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be checked.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is mIU/mL or IU/L: 1 mIU/mL = 1 IU/L.

Specimen dilution

Samples with hCG concentrations above the measuring range can be diluted with HCG sample diluent. The recommended dilution ratio is 1:100. The concentration of the diluted sample must be > 100 mIU/mL. After manual dilution, multiply the result by the dilution factor.

【Biological reference interval】

According to 815 cases (male, 130; non-pregnant and post-menopausal female, 143; pregnant female, 542) of human serum samples in Guangdong hospitals, P. R. China, the percentile 2.5 to 97.5 draws a reference range for male, non-pregnant and postmenopausal women was 0.5~5.3 mIU/mL; and for women in gestation

lists as follow (weeks of pregnancy start from last menstruation):

Weeks of gestation	Reference range (IU/L)
1-10	51.3~215298
11-15	32921~219788
16-24	8238~75291
25-40	5563~65170

Each laboratory should investigate transferability of the reference range to its local population and if necessary determine its own reference ranges.

【Result Interpretation】

In interpreting the results, the patient's overall clinical situation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.

The measuring range of the kit is 0.5~10000 IU/L. Values below lower detection limit are reported as < 0.5 IU/L. Values above the measuring range are reported as > 10000 IU/L (or up to 1000000 IU/L for 100-fold diluted samples).

There is no high-dose hook effect at hCG concentration 205000 mIU/mL.

When samples contain the bilirubin concentration of 10 mg/dL or less, lipid concentration of 1400 mg/dL or less, concentration of biotin 20 ng/mL or less, hemoglobin concentration of 500 mg/dL or less, HAMA concentration at 60.4 ng/mL or less, the interference bias of determination results deviation within ± 10%. The kit is undisturbed by rheumatoid factor (800 IU/mL).

【Analytical Performance】

Lower limit of measurement

The lower detection limit is 0.5 IU/L (repeatibility study, n = 20).

Precision

The relative bias of measuring trueness control materials should fall between 0.90 and 1.10.

Linearity

The correlation coefficient (r) was not less than 0.9900 in the interval of 3.0~8000 IU/L.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than 7.5%.

Between-lot precision

The coefficient of variation (CV) is less than 10%.

Analytical specificity

Test the following analog-additive blank samples, the results were lower than 0.5 IU/L.

Analog	Concentration
LH	400 IU/L
FSH	400 IU/L
TSH	400 mIU/L

【Method Comparison】

A comparison of Lifotronic HCG assay with Roche HCG STAT assay using clinical samples gave the following correlation:

y=0.9924x+3.7813 Pearson's r=0.9987 (concentration range: 1.0~8000 IU/L)

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

When using the kit, it's necessary to comply with regulations in the laboratory.

All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.

Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.

Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation. It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations. The preservative ProClin™ 300 contains 3% 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), will be harmful if inhalation, contact with skin and/or swallow, and may be toxic to aquatic organisms; thus, proper personal protection should be adopt while handling the reagent, and abandoned reagents should be dealed with in compliance with local regulations.

【Symbol】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use by		In vitro diagnostic medical device
	Lot number		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limitation		This way up
	Authorized representative in the European Community		Indicates this device is in compliance with Europe Directive.
	Catalogue number		

【Reference】

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5. Yaron Y, Ochshorn Y, Heifeitz S, *et al.* . Fetal Diagn Ther, 2002, 17(6): 352-356.
6. Lepage N, Chitayat D, Kingdom J, *et al.* AmJ Obstet Gynecol, 2003, 188(5): 1354-1359.

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