
HUMAN CHORIONIC GONADOTROPIN (HCG)

CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL0106-2

INTENDED USE

The Autobio human chorionic gonadotropin (hCG) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of hCG concentration in human serum.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a sialoglycoprotein with a molecular weight of approximately 46,000 daltons.¹ hCG is initially secreted by the trophoblastic cells of the placenta shortly after implantation of the fertilized ovum into the uterine wall.^{2,3} The rapid rise in serum levels of hCG after conception makes it an excellent marker for early confirmation and monitoring of pregnancy.

The placental hormone, hCG, is similar to luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). All are glycoproteins consisting of two noncovalently bound dissimilar subunits, designated alpha and beta, with attached carbohydrate sidechains. The alpha subunits of these glycoproteins are very similar. In contrast, the beta subunit portions determine the biological and immunochemical specificities.^{4,5} The beta subunits of hCG and LH exhibit considerable homology in amino acid content. Amino acid residues specific for the beta subunit of hCG confer the immunochemical specificity.⁶

According to the literature, circulating hCG typically reaches levels of approximately 2,000mIU/mL a month after conception. In the normal second-trimester maternal sera, the level of intact hCG ranges from 20,000 mIU/ml to 50,000mIU/ml. After the third month a gradual decline sets in. Following delivery, the hCG level normally undergoes rapid descent, reaching nonpregnant concentrations some two weeks later.

Ectopic pregnancies and pregnancies terminating in spontaneous abortion tend to have lower than normal circulating hCG levels, while somewhat higher levels are often seen in multiple pregnancies.

PRINCIPLE OF THE TEST

The hCG CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The hCG molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The related light unit (RLU) of the reaction is proportional to the concentration of hCG present in the sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to human chorionic gonadotropin (anti-hCG MAB) (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-hCG MAB in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 10, 25, 50, 100, 500, and 1000mIU/ml hCG in Stabilizing Buffer (6 vials, 0.5ml/ea)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200 μ l, 100-1000 μ l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent
5. Washer for microplate
6. Quality control specimens
7. Incubator

-
8. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2~8°C upon receipt. The test kit may be used throughout the expiration date of the kit (6 months from the date of manufacture). Refer to the package label for the expiration date.
2. Reconstituted standards should be stored sealed at 2~8°C. Reconstituted standards should be used within 14 days and be frozen at -20°C for long term storage. Avoid repeated freezing and thawing of the standards. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2~8°C. For longer storage, freeze the specimens at -20°C. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18~25°C) prior to use.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2~8°C.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 20µl of hCG standards, specimens, and controls into appropriate wells.
2. Dispense 100µl of enzyme conjugate reagent to each well. Mix gently for 30 seconds.
3. Incubate at 37°C for 60 minutes.
4. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300µl.
5. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.

- Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU value of each well.

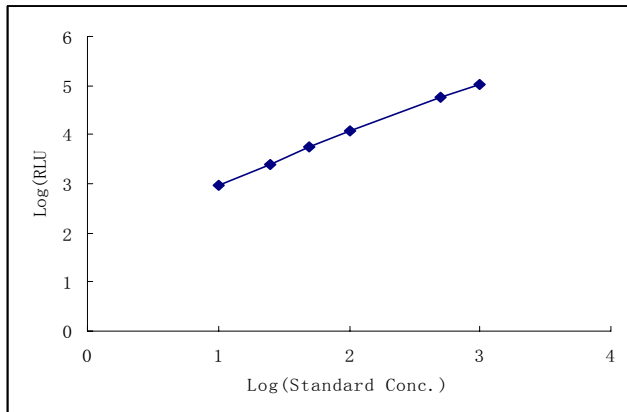
CALCULATION OF RESULTS

- Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
- On logarithmic graph paper plot the \log_{10} RLU (ordinate) obtained from each reference standard against the common logarithm of corresponding concentration of hCG in mIU/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with a curved line.
- Read the concentration for each control and sample by interpolating on the calibration curve.
- Any diluted specimens must be corrected by the appropriate dilution factor.
- Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

hCG(mIU/ml)	RLU
10	916.05
25	2541.03
50	5785.45
100	11807.9
500	58514.1
1000	105607.2



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. Approximately 95% of the normal healthy population has hCG levels less than 10mIU/ml.

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio hCG CLIA kit is not higher than 2.5mIU/ml.

B. Specificity

No interference was detected with the performance of Autobio hCG CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
TSH	500 μ IU/ml
LH	500mIU/ml
FSH	500mIU/ml

C. Precision

a. Intra-assay precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	88.23	6.15	6.97
High titer	20	279.65	12.06	4.31

b. Inter-assay precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV(%)
Low titer	10	91.27	7.25	7.94
High titer	10	281.64	16.34	5.80

D. High Dose Hook Effect

No hook effect occurred with HCG concentration up to 100000mIU/ml.

E. Accuracy

For 215 specimens in the range of 5mIU/ml to 1000mIU/ml, the correlation between the Autobio hCG CLIA kit and DPC IMMULITE® 1000 assay was as follows:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
DPC IMMULITE® 1000	215	$y = 0.9736x - 0.8972$	0.979

LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER



CONSULT INSTRUCTIONS FOR USE

REFERENCES:

1. Felig P, Baxter JD, Broadus AE, Frohman LA., eds. Endocrinology and Metabolism (2nd Ed.), New York: McGraw-Hill Book Co. 1987:253.
2. Braunstein GD, Razor J, Adler D, Danzer H, Wade ME. Serum Human Chorionic Gonadotropin Levels Throughout Normal Pregnancy. Am J Obstet Gynecol 1976;126:678-81.
3. Brody S, Carlstrom G. Immunoassay of Human Chorionic Gonadotropin in Normal and Pathologic Pregnancy. J Clin Endocrinol Metab 1962;22:564-74.
4. Swaminathan N, Bahl OP. Dissociation and Recombination of the Subunits of Human Chorionic Gonadotropin. Biochem Biophys Res Commun 1970;40:422-7.
5. Human Reproduction Unit, World Health Organization, Geneva, Switzerland. Assay of Protein Hormones Related to Human Reproduction: Problems of Specificity of Assay Methods and Reference Standards. Acta Endocrinol 1972;71:625-37.
6. Ross GT. Clinical Relevance of Research on the Structure of Human Chorionic Gonadotropin. Am J Obstet Gynecol 1977;129:795-805.

For order and inquires, please contact

AUTOBIO DIAGNOSTICS CO., LTD.

ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area,
Zhengzhou , China 450016

Tel: +86-371-67985313 Fax: +86-371-67985804

Web: www.autobio.com.cn