IVD

HCG ELISA

Catalog No. E0106-2

96 tests

INTENDED USE

Autobio HCG ELISA is intended for the quantitative determination of human chorionic gonadotropin (HCG) concentration in human serum or urine samples.

INTRODUCTION

Human chorionic gonadotrophin (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. 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.

PRINCIPLE OF THE TEST

Autobio HCG ELISA is based on the sandwich ELISA method. Microwells are first pre-coated with anti-HCG. The test sample is added to the wells then incubated. If human HCG is present in the sample, it will bind to the anti-HCG on the well. The wells are then washed to remove unbound materials. Thereafter, anti-HCG conjugated to horseradish peroxidase (HRP) is added. The conjugate will bind immunologically to the HCG on the well, resulting in the HCG molecules being sandwiched between the solid phase and the conjugate. After incubation, the wells are again washed to remove unbound materials. Substrate solution and chromogen solution are added and incubated, resulting in the development of a blue color. Add the stop solution, and the color turns to yellow and is measured spectrophotometrically at 450nm. The concentration of HCG is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED

1. Coated Wells: microplate with murine anti-HCG MAb coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: murine anti-HCG MAb conjugated to HRP in stabilizing buffer (1 vial, 12.0 mL)
3. Reference Standards: 0, 25, 50, 100, 200, 400 mIU/mL HCG in human serum with preservatives. (6 vials, freeze-dried)
4. Wash Fluid Concentrate: PBS-Tween (1 vial, 16.0 mL, 30×)
5. Substrate Solution: hydrogen peroxide (1 vial, 7.5 mL)
6. Chromogen Solution: 3, 3′, 5, 5′-tetramethylbenzidine (TMB) (1 vial, 7.5 mL)
7. Stop Solution: 1.0 M H_2SO_4 (1 vial, 7.5 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper

6. Incubator
7. Disposable reagent troughs
8. Instrumentation
    1. Automated microplate stripwasher
    2. Microplate reader

    or

    3. Fully automated microplate processor

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 – 8℃ upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Reference Standards should be stored sealed at 2 – 8℃. The remaining Reference Standards opened should be used within 30 days and be frozen at -20℃ for long term storage. Avoid multiple freeze-thaw cycles of Reference Standards.
3. 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, or until the expiration date, whichever is earlier, provided it is stored as prescribed above.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE

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℃. Specimens going to be stored or transported for more than 48 hours must be stored frozen (- 20℃ or lower). Avoid multiple freeze-thaw cycles. After thawing, ensure samples are thoroughly mixed and brought to room temperature before being assayed.

PRECAUTIONS AND WARNINGS

1. for *in vitro* diagnostic use only
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. Handling of reagents, serum specimens should be in accordance with local safety procedures.
4. Reference 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 bovine spongiform encephalopathy (BSE) has not been reported. Nevertheless, the Reference Standards and components containing animal substances should still be treated as potentially infectious.
5. Avoid any skin contact with all reagents. Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
6. 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) before use. All reagents should be mixed through gently inverting or swirling prior to use. Do not induce foaming.
2. Reconstitute each freeze-dried Reference Standard with 1.0 mL distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted Reference Standards should be stored sealed at 2 – 8℃. Reconstituted Reference Standards should be used within 14 days and be frozen at -20℃ for long term storage.
3. Dilute Wash Fluid Concentrate 30 folds with distilled water.

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 pipetting is used, since pipetting of all Reference Standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting 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 falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 100 μL of each Reference Standard and sample into appropriate wells.
2. Incubate at 37℃ for 10 minutes.
3. Wash the microplate 5 times. Strike the plate sharply onto absorbent paper to remove residual water droplets at the end of the last wash cycle. The use of an automated microplate stripwasher is recommended.
4. Add 100 μL Enzyme Conjugate Reagent into each well.
5. Incubate at 37℃ for 10 minutes.
6. Repeat step 3.
7. Add 50 μL Substrate Solution, then 50 μL Chromogen Solution into each well. Gently mix and incubate for 5 minutes at room temperature in the dark.
8. Add 50 μL Stop Solution to each well.
9. Gently mix for 10 seconds to ensure that the blue color completely turns to yellow.
10. Read absorbance at a wavelength of 450 nm in a microplate reader within 10 minutes.

CALCULATION OF RESULTS

1. Calculate the mean values from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Construct a standard curve by plotting the mean absorbance obtained for each Reference Standard against its concentration in mIU/mL on logrithmic graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance for each sample, determine the corresponding HCG concentration in mIU/mL from the standard curve. Any diluted sample must be corrected by the appropriate dilution factor.

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 standard curve.

HCG (mIU/mL)	Absorbance (450 nm)
5	0.248
10	0.406
20	0.713
40	1.302
80	2.368

REFERENCE NORMAL RANGE

Each laboratory should establish its own reference normal range. These values are given only for guidance.

Sample Numbers（smokers）	326	**Sample Numbers（smokers）**	248
Average Value（ng/mL）	1.36	**Average Value（ng/mL）**	3.46
Standard Deviation ( )	1.82	**Standard Deviation ( )**	3.27
Normal Range (+2 , ng/mL)	5.0	**Normal Range (+2 , ng/mL)**	10.0

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

The analytical sensitivity of the assay, defined as the concentration of HCG equivalent to the mean absorbance of 20 replicates of the zero Reference Standard plus two standard deviations, is typically 12.5 mIU/mL.

2. Specificity

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

Substance	Concentration
AFP	500ng/ml
CA125	400U/ml
CA15-3	500U/ml
CA19-9	500U/ml

3. Precision

3.1. Intra-assay precision

Intra-assay precision was determined by assaying 20 replicates of each of 2 controls, low titer and high titer, respectively.

Serum	Number	Mean	SD	RSD（%）
Low	20	24.36	1.43	5.87
High	20	220.34	11.55	5.24

3.2. Inter-assay precision

Inter-assay precision was determined by assaying 2 serum pools in duplicate across 20 separate runs, using a standard curve constructed for each run.

Serum	Number	Mean	SD	RSD（%）
Low	20	23.87	2.35	9.84
High	20	223.06	18.85	8.45

4. High Dose Hook Effect

No interfere with HCG values up to 6000 ng/mL.

5. Accuracy

For 128 samples in the range of 5 ng/mL to 80 ng/mL, the relationship between the Autobio HCG ELISA and the Elecsys CLIA assay is described by the equation below:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Roche（ECLIA）	128	y = 1.0057x - 1.9464	0.945

LIMITATIONS

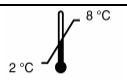
1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering 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 assay.
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

LOT	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**

	**CONTAINS SUFFICIENT FOR <n> TESTS**
	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

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4. LOKICH, J.J., ZAMCHECK, N., LOWENSTEIN, M., Sequential carcinoembryonic antigen levels in the therapy of metastatic breast cancer. Ann. Inter. Med. 89: 902, 1978.
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