BIOGENIX
INC. PVT. LTD.

hCG ELISA

Enzyme Immunoassay for Quantitative Determination of Human Chorionic Gonadotropin in Human Serum

1. SYMBOL LEGEND

IVD	In vitro diagnostic medical device	LOT	Batch code
REF	Catalogue number		Manufacturer
	Use by		Consult operating instructions
	Date of manufacture		Biological risks
	Temperature limitation	CONJ	Conjugate
	Caution, consult accompanying documents	SUB	Substrate
MP	Coated microplate (96 wells)	STOP	Stop solution
WASH P 20X	Wash solution, 20X concentrated	OD	Optical density
CAL	Calibrators	DIL	Sample diluent
CONTROL	Control	H2O	Deionized or distilled water
	Contains sufficient for <n> tests		Irritant
		Warning	
RCNS	Reconstitute with specified volume of liquid	TRIAL 5000X	Trial

2. INTENDED USE

hCG Elisa kit is provided for the **quantitative determination of human chorionic gonadotropin (hCG) in human serum**.

hCG is a glycoprotein hormone that consists of two subunits (α and β). Quantitative measurement of hCG is regarded as the most reliable indicator for early diagnostics of pregnancy.

Alteration in serum level of hCG in pregnant women is an important method for prenatal diagnostics of some inborn diseases. Besides, this method is widely used in obstetrics for diagnostics of multiple pregnancy, ectopic pregnancy and the threatening abortion. Though hCG presence in serum is usually associated with normal pregnancy, increased level of hCG may be also detected in patients with teratogenic carcinomas or throphoblastic neoplasias. Less frequently hCG concentration is increased in the case of ectopic synthesis due to testis, breast, intestinal, lung or prostate cancer. hCG concentration in patients with some particular forms of cancer may exceed 100000 IU/L. Measurement of hCG is an important method for diagnostics and monitoring of such diseases.

3. PRINCIPLE OF TEST

hCG Elisa kit is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of hCG β-subunit One of these antibodies is conjugated with horseradish peroxidase (HRP); the other is coated onto the inner surface of microwells. hCG molecules from the serum sample bind to both immobilized antibody and anti-hCG-peroxidase conjugate. Then the wells are washed with wash solution to remove any material not

bound on the inner surface of the wells. Quantity of the bound conjugate is directly proportional to hCG level in tested sample.

During the incubation with TMB solution the colour is developing. The intensity of the colour is directly proportional to hCG concentration in specimens. hCG concentration in the patient sample is read from a standard curve that is processed in each assay.

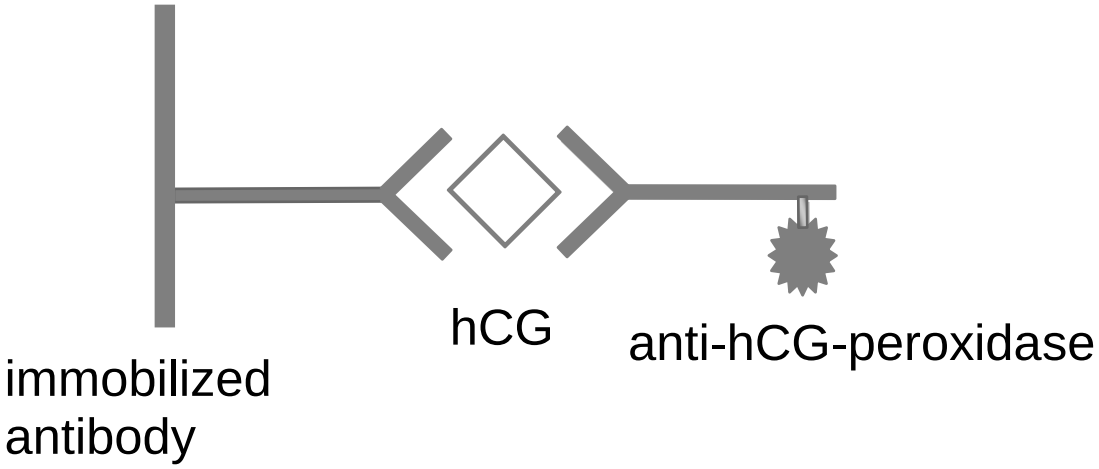

Fig. 1. Assay scheme

4. STORAGE AND STABILITY OF THE KIT

The expiration date of the kit is printed on the box label; the expiration date for each component is printed on the respective label.

hCG Elisa kit should be stored at 2-8°C upon receipt, preferably in the original kit box, until the expiration date. Storage at 25°C is allowed but for no more than 5 days.

Shelf life of the kit is 18 months.

After initial opening the kit is stable until the expiration date if stored at 2-8°C.

If used in several separate experiments, after initial opening kit contents should be stored as follows:

- unused strips: in a firmly closed resealable zipper bag at 2-8°C until the expiration date;
- vials with conjugate, sample diluents, calibrators and control (ready-to-use): at 2-8°C until the expiration date; vials with calibrators and control (reconstituted): at 2-8°C for no more than 1 month after opening;
- vials with concentrated Trial, concentrated wash solution and stop solution: at 2-8°C until the expiration date;
- vial with substrate: at 2-8°C until the expiration date, protected from light;
- wash solution and Trial solution prepared for use: at room temperature (18-25°C) for no more than 5 days or at +2…+8°C for no more 4 week, in a firmly closed bottle.

5. SAMPLE COLLECTION AND STORAGE

Collect blood by venipuncture. After clotting, the serum is separated by centrifugation.

Do not use plasma, haemolyzed (bright red) or lipemic (milky) serum samples as well as samples containing sodium azide as preservative.

Store serum samples at 2-8°C for no more than 2 days. Aliquot and freeze samples for longer storage (–20°C and lower). Avoid repeated freezing.

6. EXPECTED VALUES

Serum samples collected between 9 and 11 a.m. from 200 apparently healthy people (both males and females) at the age of 18–40, were assayed with **hCG Elisa kit**. hCG concentration range was 0–10 IU/L.

Expected hCG concentrations in sera of pregnant women at different terms of normal gestation and recommended sample dilutions are shown in Table 1. These limits should be considered as guidelines only.

It is highly recommended for each laboratory to determine its own reference range of hCG concentrations.

7. QUALITY CONTROL

It is recommended to use control samples according to the state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

8. REAGENT PREPARATION

Allow all the reagents to reach room temperature (18-25°C), and then thoroughly stir.

MP Keep **microplate** at room temperature for at least 30 minutes before opening the bag. Place required number of strips onto strip holder. Place unused strips into the resealable zipper bag and reseal duly.

CAL **CONTROL** **Calibrators and Control**

Liquid calibrators and control are ready to use.

Prepare lyophilized calibrators and control as follows. Gently tap on the vial caps to knock off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 mL of distilled or deionized water to each vial with lyophilized calibrators and control, close vials with the corresponding caps and leave for 10 min at room temperature without stirring. Then stir gently avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at

room temperature stirring gently periodically. Make sure that no dry matter is left on the caps and walls of the vials.

WASH | **P** Prepare required volume of **wash solution** by dilution of the concentrate 20-fold with distilled or deionized water. For example:

5 mL of **WASH** | **P** **20X** + 95 mL of water.

Mix thoroughly, avoid foaming.

SUB Protect **substrate** from direct light.

9. SAMPLE PREPARATION

Allow samples to reach room temperature (18-25°C). Stir samples gently in order to ensure homogeneity.

If the expected hCG concentration in the sample is higher than in calibrator 5, the sample should be diluted with sample diluent 20-fold, 400-fold or 2 000-fold in concordance with instructions for use for manually before analysis (see the Table 1 for recommended dilution). The example of manual sample dilution as follows:

Sample 1 (20-fold dilution): 380 µL of sample diluent **DIL** + 20 µL of serum sample;

Sample 2 (400-fold dilution): 380 µL of sample diluent **DIL** + 20 µL of the Sample 1;

Sample 3 (2 000-fold dilution): 320 µL of sample diluent **DIL** + 80 µL of Sample 2.

Vortex or mix thoroughly.

Gestation period after the LMP (weeks)	Median (IU/L)	Reference range of hCG concentration (IU/L)	Recommended sample dilution
2	150	50–300	Without dilution
3–4	2 000	1 500–5 000	1: 20
4–5	20 000	10 000–30 000	1:400
5–6	50 000	20 000–100 000	1:400
6–7	100 000	50 000–200 000	1:400 and 1:2 000
7–8	80 000	40 000–200 000	1:400 and 1:2 000
8–9	70 000	35 000–140 000	1:400
9–10	65 000	32 500–130 000	1:400
10–11	60 000	30 000–120 000	1:400
11–12	55 000	27 500–110 000	1:400
13–14	50 000	25 000–100 000	1:400
15–16	40 000	20 000–80 000	1:400
17–21	30 000	15 000–60 000	1:400

10. PERFORMANCE CHARACTERISTICS OF THE ASSAY

10.1. Calibration - Traceability

hCG Elisa kit was calibrated against the WHO 3rd International Standard 75/537.

10.2. Specificity

Cross-reactivity of both monoclonal antibodies used in the assay with TSH, FSH and LH is negligible.

10.3. Analytical Sensitivity

Analytical sensitivity of **hCG Elisa kit kit**, i.e. concentration that can be distinguished from zero calibrator, is 5 IU/L. It is defined as mean OD of 10 replicates of calibrator 0 plus 2SD.

10.4. Measurement Range

hCG Elisa kit was validated for measurement of hCG concentration within the concentration diapason (without dilution) of 5-500 IU/L.

10.5. Hook Effect

High dose hook effect was determined by spiking calibrator 0 matrix with antigen For **hCG Elisa kit** no **high dose hook effect** was detected for concentrations up to 400 000 IU/L.

10.6. Intra- and Inter-Assay Variation

For **intra-assay CV** determination, 8 serum samples were assayed in 9 replicates each. The results are shown below.

Sample	Mean hCG concentration, IU/L	Intra-assay CV	
		SD	CV, %
HS 1	13.0	0.81	6.2
HS 2	22.3	1.69	7.6
HS 3	45.5	2.91	6.4
HS 4	62.8	3.71	5.9
HS 5	98.9	5.14	5.2
HS 6	164	7.54	4.6
HS 7	384	15.4	4.0
HS 8	434	18.7	4.3

For **inter-assay CV** determination, 8 serum samples were assayed 3 times by different operators with 1-week interval.

Each specimen was assayed in 9 replicates. The results are shown below.

Sample	Mean hCG concentration, IU/L			Inter-assay CV	
	1 assay	2 assay	3 assay	SD	CV, %
HS 1	19.3	16.4	18.6	1.51	8.4
HS 2	54.0	58.2	51.1	3.57	6.6
HS 3	112	127	121	7.6	6.3
HS 4	157	164	148	8.2	5.3
HS 5	243	251	247	4.2	1.7
HS 6	354	340	369	14.4	4.1
HS 7	412	447	405	22.4	5.3
HS 8	436	470	459	17.4	3.8

11. LIMITATION OF THE METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. To state a diagnosis, the physician is supposed to consider all the available clinical and laboratory findings.

12. SAFETY PRECAUTIONS

- **This kit is for in vitro diagnostic use only.** Operator should follow the manual closely in order to ensure reliable data. The manual is valid for the present kit only, within the listed composition. Any substitution of kit components is not allowed by CE regulations.
- Do not use kit or its components after the expiration date indicated on the label. Take into account stability period for reconstituted reagents.
- Do not mix or use together reagents from different lots of the kit except substrate, stop solution and wash solution.

- Do not use substrate, stop solution and wash solution supplied by other vendors.
- Note that stop solution is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected area thoroughly with plenty of water and seek medical advice.
- Take into account the following common procedural notes:
    - always pipette reagents into wells immediately after washing procedure;
    - avoid contamination of the solutions;
    - in case of partial use of the kit, dispense only required volume of the reagent into the tray;
    - do not pour unused reagents back into the original vials;
    - avoid exposure to direct sunlight during incubations;
    - always pipette reagents in the same order to minimize reaction time differences between wells; the total dispensing time for the calibrators, control and samples must not exceed 15 min;
    - the incubation temperature for all the immunological reactions must be kept at 37°C;
    - do not touch the bottom of the wells.
    - calibrators should be measured in each separate assay. It is also recommended to measure each time hCG concentration in the control.
- TMB solution should be colourless. Light colouring of solution is admissible. Avoid direct exposure of substrate to sunlight.
-  Source materials of human origin used for kit components preparation were tested and found negative for

HBsAg, anti-HIV and anti-HCV antibodies. However, none of known laboratory test guarantees absence of these viral agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.

-  After usage strips, calibrators, control, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving. Instead of autoclaving pipette tips may be sterilized by disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other components of the kit should be discarded into conventional garbage.

-  During manual washing procedure do not discard the contents of the wells directly to drainage. Use a container with disinfectant solution.

-  As the kit contains potentially hazardous material, the following precautions should be taken:
  - do not smoke, eat or drink while performing the assay;
  - always use protective gloves;
  - never pipette material by mouth;
  - in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.
- GLP including all general and individual regulations should be applied for the kit usage.

 As the kit contains irritant (CONJ, DIL, CAL, CONTROL), the following precautions should be observed:

- P261 - Avoid breathing spray;
- P272 - Contaminated work clothing should not be allowed out of the workplace;
- P280 - Wear protective gloves/protective clothing/eye protection;
- P302+P352 - IF ON SKIN: Wash with plenty of soap and water;
- P333+P313 - If skin irritation or rash occurs: Get medical advice/attention;
- P363 - Wash contaminated clothing before reuse;
- P501 - Dispose of contents/container in accordance with national regulation.

Precautionary statements according to Regulation EC № 1272/2008.

13. KIT CONTENTS

13.1. Material Provided

MP	**Microplate:** 12 breakable 8-well strips (total 96 wells) coated with anti-hCG monoclonal antibodies	1 pcs
CONJ	**Conjugate:** solution contains anti-hCG monoclonal antibodies conjugated with HRP	18 mL, ready to use
0–5 **CAL**	**hCG calibrators:** protein-based solution or lyophilized preparations containing known hCG concentrations – 0; 15; 50; 125; 250; 500 IU/L. The concentrations of calibrators may be different for schemes with or without shaking. For exact hCG concentrations, see **Quality Control Sheet** 	6 vials, 0.5 mL each; ready to use or lyophilized preparations
CONTROL	**hCG control:** protein-based solution or lyophilized preparation containing known hCG concentration. The range of hCG concentration may be different for schemes with or without shaking. For exact range of hCG concentration see **Quality Control Sheet** 	0.5 mL, ready to use or lyophilized preparations
SUB	**Substrate (TMB solution):** 3,3',5,5'-tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide	14 mL, ready to use
WASH **P** **20X**	**Wash solution P, 20X concentrated:** surfactant in buffered saline, sufficient for preparation of 280 mL of solution	2x14 mL, concentrated
STOP	**Stop solution:** 1N HCl solution	14 mL, ready to use
DIL	**Sample diluent**	20 mL, ready to use

13.2. Equipment and Materials Required but not provided

- 1-channel calibrated variable precision pipettes, with disposable tips;
- 8-channel calibrated variable precision pipette, with disposable tips;
- microplate incubator/shaker (37°C, shaking speed 500–800 rpm);
- manual or automatic equipment for rinsing wells;
- calibrated microplate reader (450 nm);
- vortex tube mixer;
- deionized or distilled water;
- graduated beaker and cylinder of appropriate volume;
- latex or plastic gloves;
- trays for pipetting reagents with 8-channel pipette;
- disinfectant;
- absorbent material (for manual wash).

13.3. Test Procedure

hCG Elisa kit is designed for 96 tests. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 blank (OD of TMB solution) in duplicates, provided that all the strips are used simultaneously.

13.3.1. Assay Procedure

13.3.1.1. Protocol with shaking (see assay scheme, section 13.5)

A. Pipette**:**

- **150 µL** of **conjugate** **CONJ** into each well **except wells A1-A2 (blank);**

B. Pipette**:**

- **50 µL** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates, into the respective wells**;** **except wells A1-A2**.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for **60 minutes at 37°C while shaking (500–800 rpm).**

D. Wash 5 times, as described in section 13.3.2.

E. Pipette **100 µL** of **substrate** **SUB** into each well (including blank); incubate strips **at room temperature** (18-25°C) **in the dark for 15-30 minutes**, depending on the colour intensity, or **10 minutes while shaking (500-800 rpm) at 37°C**.

F. Pipette **100 µL** of **stop solution** **STOP** into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within 20 minutes**.

13.3.1.2. Protocol without shaking (see assay scheme, section 13.6)

A. Pipette**:**

- **150 µL** of **conjugate** **CONJ** into each well **except wells A1-A2 (blank);**

B. Pipette**:**

- **50 µL** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates, into the respective wells**; except wells A1-A2**.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for **90 minutes at 37°C (pre-shake for 1-2 minutes at room temperature)**.

D. Wash 5 times, as described in section 13.3.2.

E. Pipette **100 µL** of **substrate** **SUB** into each well (including blank); incubate strips **at room temperature** (18-25°C) **in the dark for 15-30 minutes**, depending on the colour intensity.

F. Pipette **100 µL** of **stop solution** **STOP** into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within 20 min**.

13.3.2. Wash Procedure

It is advisable to use an automatic microplate washer set at 5 wash cycles and a volume of 300 µL of wash solution per well per cycle.

If an automatic washer is not available, the wash procedure can be carried out manually as follows:

- remove the contents of the wells into a container with disinfectant;
- dispense 300 µL of wash solution, prepared according to section 8, into each well, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 5 times;
- strike the wells sharply on absorbent material to remove any liquid residue.

13.4. Data Processing

If the reader cannot be adjusted to zero using the substrate blank in wells A1-A2, subtract mean OD value of wells A1-A2 from all OD values before further calculations.

Example:

OD (Cal 5) measured = 2.28 and OD (blank) = 0.06;

OD (Cal 5) calculated = 2.28 – 0.06 = 2.22

13.4.1. Data Reliability (for OD Measured at 450 nm)

The data should meet the following criteria:

- average blank OD (in wells A1-A2) ≤0.100;
- average OD of Cal 5 ≥1.5 (after blank subtraction);
- control’s concentration must fall within the acceptability range that is shown on the vial label.

If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

13.4.2. Quantitative Determination

Specialized software for quantitative determination is recommended. Mean OD values of the calibrators at 450 nm versus their respective hCG concentrations using 4PL or 5PL fit (see typical standard curve, fig. 2). Calculate concentration of hCG in samples using standard curve.

Any extrapolation of the standard curve to hCG concentration above the nominal value of calibrator 5 is forbidden. In this case the sample should be diluted 20-; 400- or 2 000-fold with sample diluent and re-tested. Multiply the measured concentration of pre-diluted samples by corresponding dilution factor.

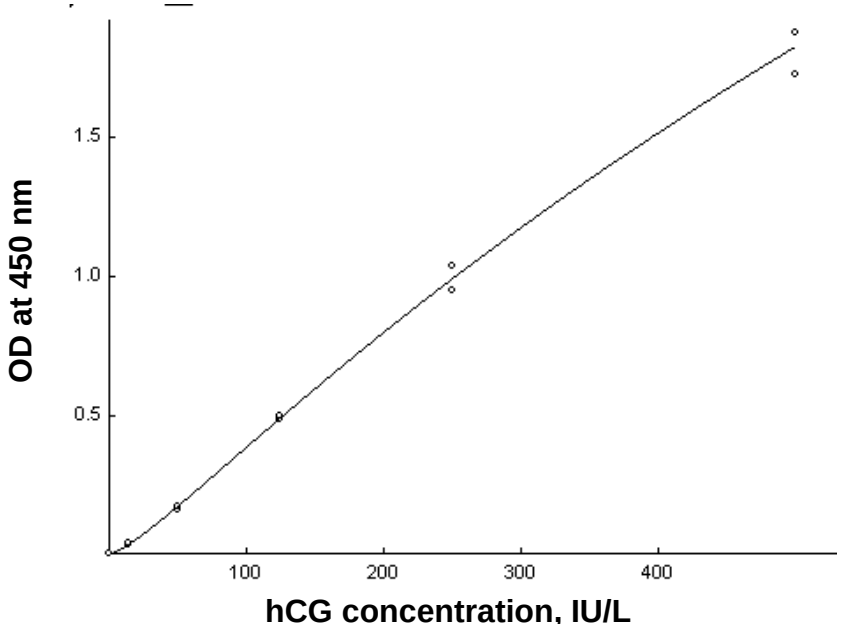

Fig. 2. Example of typical standard curve.
Do not use for evaluation of real assay data!

13.5. Assay scheme with shaking

Wells / Reagents	«Blank»	CAL / CONTROL	Samples
CONJ	–	150 µL	150 µL
CAL / **CONTROL**	–	50 µL	–
Samples	–	–	50 µL
Incubation No.1	60 min, 37°C, 500–800 rpm		
WASH **P** (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, 18-25°C, in the dark		
	10 min, 37°C, 500–800 rpm		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, 18-25°C		
OD measuring	450 nm		
Calculations	Corresponding software		

13.6. Assay scheme without shaking

Wells / Reagents	«Blank»	CAL / CONTROL	Samples
CONJ	–	150 µL	150 µL
CAL / **CONTROL**	–	50 µL	–
Samples	–	–	50 µL
Incubation No.1	90 min, 37°C (pre-shake for 1-2 minutes at room temperature)		
WASH **P** (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, 18-25°C, in the dark		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, 18-25°C		
OD measuring	450 nm		
Calculations	Corresponding software		

BIOGENIX INC. PVT. LTD.
Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road, Lucknow - 226008 (U.P.), India
Email: biogenix2007@yahoo.com, info@biogenixinc.com
Website: www.biogenixinc.com
Customer care no: +919140971443