

**REF**

GD7090 00

# HCG- $\beta$

Enzyme-immunoassay for the quantitative  
determination of human chorionic gonadotropin  
in serum or plasma

**IVD**

## INDICATION

HCG is a glycoprotein hormone secreted in pregnancy. It is produced by the embryo soon after conception and, later, by the placenta. HCG consists of two subunits:  $\alpha$  subunit identical to that of thyrotropin (TSH), luteinising hormone (LH), follicle-stimulating hormone (FSH), and  $\beta$  subunit (HCG- $\beta$ ) HCG specific determining its function.

Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production that is critical for pregnancy. HCG also affects the immune tolerance of the gestation.

Pregnancy tests measure the levels of HCG in the blood or urine to indicate the presence or absence of an implanted embryo. HCG is also considered as a tumor marker, as it is secreted by some cancers including teratomas and choriocarcinomas. Otherwise, elevated levels cannot prove the presence of a tumor, and low levels do not exclude its absence.

## PRINCIPLE OF THE ASSAY

This test is based on simultaneous binding of HCG- $\beta$  to two monoclonal antibodies, one immobilized on microwell plates, the other conjugated to horseradish peroxidase (HRP). After incubation and subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate solution. The developed colour, detected at 450 nm, is directly related to the quantity of HCG- $\beta$  present in the specimen.

HCG- $\beta$  concentration in the sample is calculated based on a series of standards.

## KIT CONTENT

- 1. Reagent A – Microplate**  
12x8 strips.  
8 wells breakable strips, coated with anti-HCG- $\beta$  monoclonal antibody. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.
- 2. Reagent B1 – Enzymatic Tracer 100x**  
1 vial of 0.300 ml.  
Concentrated human anti-HCG- $\beta$  monoclonal antibody, conjugated with Horseradish peroxidase (HRP).
- 3. Reagent B2 – Incubation Buffer**  
1 vial of 50 ml.  
Phosphate buffer 50 mM, BSA 1g/l, pH 7.4
- 4. Reagent C – Washing Solution 50x**  
1 vial of 20 ml.  
Concentrated solution to be diluted 1:50 with distilled water. It contains NaCl 9 g/l, Tween 20 1 g/l.
- 5. Reagent D/E – Chromogen/Substrate**  
1 vial of 12 ml.  
Ready to use solution containing Tetramethylbenzidine (TMB) 0.25 g/l.  
**Avoid any skin contact and light exposure.**
- 6. Reagent F – Stop Solution**  
1 vial of 12 ml.  
Ready to use solution containing Sulphuric acid 0.15 M.  
**Avoid any skin contact.**
- 7. HCG- $\beta$  Standards**  
6 vials of 1 ml each.  
Ready to use liquids containing approximately HCG- $\beta$  at the following concentrations:  
**S<sub>0</sub>: 0 mIU/ml, S<sub>1</sub>: 1 mIU/ml, S<sub>2</sub>: 5 mIU/ml,**  
**S<sub>3</sub>: 20 mIU/ml, S<sub>4</sub>: 100 mIU/ml, S<sub>5</sub>: 400 mIU/ml.**  
(Calibrated against WHO 1<sup>st</sup> IRP 75/551 standard)  
Actual concentrations to be used for calculation are stated on the labels of the vials.
- 8. Cardboard sealers**  
2 cardboard sealers to be used to cover the plate during the incubations.
- 9. Package insert:** instruction for use GD7090 00 it/ing.

## MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

## STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

## AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000  $\mu$ l
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm (620- 630 nm).
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300  $\mu$ l.

## SAMPLES

Fresh sera or plasma can be indifferently used. Samples can be stored at 2-8 °C for a maximum of two days, for longer storage the specimen should be frozen at -20 °C. Avoid repeated freezing and thawing. Highly lipemic, hemolysed or microbiologically contaminated samples should not be used in the assay.

## REAGENTS PREPARATION

- **DILUTED TRACER:** the diluted conjugated has to be prepared immediately before use. Dilute 1:100 with Incubation Buffer (Reagent B2) and mix carefully (ex.: 10  $\mu$ l of Reagent B1 + 0.990  $\mu$ l of Reagent B2). The quantity of diluted conjugate is proportional to the number of tests. The diluted tracer is stable 3 hours at room temperature.
- **WASHING SOLUTION:** dilute 1:50 with distilled or ELISA grade water (ex.: 20 ml of reagent C + 980 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for one week at room temperature or 3 weeks at 2-8 °C. It is recommended to store diluted washing solution at room temperature for immediate use.

## WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 2-3 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 3 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

## ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards and samples to room temperature (18-30°C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blanking" of the instrument is to be carried out in the blank reagent well (well A1).

### ASSAY SCHEME

- Put the desired number of microstrips into the frame.
- If suggested analyte concentration in the sample exceeds 400 mIU/ml, dilute this sample accordingly, using Incubation Buffer (Regent B2).
- Follow the scheme:

	Microplate wells coated with anti-HCG- $\beta$ antibody			
	REAGENTS	Blank	Standard	Sample
Immunological reaction	Standard	-	25 $\mu$ l	-
	Sample	-	-	25 $\mu$ l
	Diluted tracer	-	100 $\mu$ l	100 $\mu$ l
	- Cover the strips with cardboard sealer - Incubate <b>60 minutes at room temperature</b> (22-28 °C)			
Washing	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 3 times with 200 $\mu$ l of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
	- Cover the strips with cardboard sealer - Incubate <b>15 minutes at room temperature</b> (22-28 °C), avoiding light exposure			
	Reagent F (Stop Solution)	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
	Read the absorbance of each well against Blank at 450 nm (and 620-630 nm)			

### QUALITY CONTROL

It is recommended, in each analytical run, to use control sera with known HCG- $\beta$  values, to check the correspondence of the obtained results with those expected and consequently validate the data.

### CALCULATION OF RESULTS

- Calculate the mean of the absorbance ( $E_m$ ) for each point of the standard curve (S0 - S5) and of each sample.
- Plot the mean value of absorbance of the standards ( $E_m$ ) against proper HCG- $\beta$  concentrations. Draw the best-fit curve through the plotted points. (Ex.: four parameter logistic or sigmoid).
- Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in mIU/ml.
- If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the standards fall within 10% of the assigned concentrations

### EXPECTED VALUES

From data obtained by Minias Globe Diagnostics the following reference ranges are suggested:

Normal female: < 8.0 mIU/mL

Pregnancy

1 <sup>st</sup> week:	3 - 100 mIU/ml
2 <sup>nd</sup> week:	10 - 1.000 mIU/ml
3 <sup>th</sup> week:	100 - 10.000 mIU/ml
4 <sup>th</sup> week:	1.000 - 100.000 mIU/ml
2 <sup>nd</sup> month:	15.000 - 200.000 mIU/ml
3 <sup>th</sup> month:	10.000 - 100.000 mIU/ml

It is recommended that each laboratory establish its own reference ranges.

### ANALYTICAL PERFORMANCES

#### Sensitivity

The lowest detectable concentration of HCG- $\beta$  is 0.5 mIU/ml at the 95% confidence limit.

#### Precision

##### a. Intra Assay Variation

Within run variation was determined by 16 replicate determination of two different control sera in the same analytical run. %CV values found were < 7% according to the optical density revealed.

##### b. Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. %CV values found were < 7.5% according to the optical density revealed.

#### Recovery

The recovery of 12.5 - 25 - 50 - 100 mIU/ml of HCG- $\beta$  added to sample gave an average value ( $\pm$  SD) of 98.4%  $\pm$  5% with reference to the original concentrations.

#### Correlation with RIA

The present kit was compared to a well-established RIA method. 50 female sera were assayed with both methods. The following linear regression curve was calculated:

$$y = 0.94x - 0.02 \text{ mIU/ml} \quad r = 0.96$$

**Specificity**

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

$\beta$ HCG	100 %
hCG	4.00 %
hFSH	0.30 %
hTSH	0.02 %

**"Hook" Effect**

The present method shows no "Hook" Effect up to 250.000 mIU/ml.

**PRECAUTIONS IN USE**

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

**Waste Management**

Please refer to local legal requirements.

**REFERENCES**

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