



DCM014-11  
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## $\beta$ -HCG ELISA

for routine analysis

Direct immunoenzymatic determination of  $\beta$ -HCG in human serum or plasma

IVD



LOT

See external label

2°C 8°C



$\Sigma = 96$  tests

REF DKO014

### INTENDED USE

Eagle Biosciences [b-HCG ELISA Assay Kit](#) is an immunoenzymatic colorimetric method for quantitative determination of  $\beta$ -HCG concentration in human serum or plasma. b-HCG ELISA Assay Kit is intended for research use only and not intended for diagnostic procedures.

### 1. CLINICAL SIGNIFICANCE

Human chorionic gonadotropin (HCG) is a glycoprotein hormone secreted in pregnancy, that is made by the embryo soon after conception and later by the syncytiotrophoblast (part of the placenta). Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production that is critical for a pregnancy in humans. HCG may have additional functions, for instance it is thought that it affects the immune tolerance of the pregnancy.

Pregnancy tests measure the levels of HCG in the blood or urine to indicate the presence or absence of an implanted embryo. In particular, pregnancy tests employ an antibody that is specific to the  $\beta$ -subunit of HCG ( $\beta$ -HCG). This is important so that tests do not make false positives by confusing HCG with LH and FSH.  $\beta$ -HCG is also secreted by some cancers including teratomas and choriocarcinomas. But, elevated levels cannot prove the presence of a tumor, and low levels do not rule it out.

### 2. PRINCIPLE

Eagle Biosciences b-HCG ELISA Assay Kit for  $\beta$ -HCG is based on the simultaneous capture of HCG by a monoclonal antibody immobilized on the microplate and directed against the  $\beta$ -HCG fraction, and another monoclonal antibody conjugated with peroxidase horseradish (HRP) and directed against the fraction  $\alpha$ -HCG.

After the incubation, the bound/free separation is performed by a simple solid-phase washing.

The enzyme HRP in the bound-fraction reacts with the Substrate ( $H_2O_2$ ) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution ( $H_2SO_4$ ) is added.

The color intensity is proportional to the  $\beta$ -HCG concentration in the sample. The  $\beta$ -HCG concentration in the sample is calculated based on a calibration curve.

### 3. REAGENTS, MATERIALS AND INSTRUMENTATION

#### 3.1. Reagents and materials supplied in the kit

##### 1. Calibrators (6 vials, 1 mL each)

CAL0	REF DCE002/1406-0
CAL1	REF DCE002/1407-0
CAL2	REF DCE002/1408-0
CAL3	REF DCE002/1409-0
CAL4	REF DCE002/1410-0
CAL5	REF DCE002/1411-0

##### 2. Control (1 vial, 1 mL)

Concentration of Control is Lot-specific and is indicated on the Certificate of Analysis

REF DCE045/1403-0

##### 3. Incubation Buffer (1 vial, 50 mL)

Phosphate buffer 50 mM pH 7.4, BSA 1 g/L

REF DCE001/1401-0

##### 4. Conjugate (1 vial, 1 mL)

Monoclonal antibody direct against  $\alpha$ -HCG subunit conjugated with horseradish peroxidase (HRP)

REF DCE002/1402-0

##### 5. Coated Microplate (1 breakable microplate)

Monoclonal antibody direct against  $\beta$ -HCG subunit adsorbed on microplate

REF DCE002/1403-0

##### 6. TMB Substrate (1 vial, 15 mL)

$H_2O_2$ -TMB (0.26 g/L) (avoid any skin contact)

REF DCE004-0

##### 7. Stop Solution (1 vial, 15 mL)

Sulphuric acid 0.15 mol/L (avoid any skin contact)

REF DCE005-0

##### 8. 50X Conc. Wash Solution (1 vial, 20 mL)

NaCl 45 g/L, Tween-20 55 g/L

REF DCE006-0

#### 3.2. Reagents necessary not supplied

Distilled water.

#### 3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm, 620-630 nm)

**Note**

Store all reagents between 2-8°C in the dark.  
 Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use. Do not remove the adhesive sheets on the strips unutilised.

**4. WARNINGS**

- This b-HCG ELISA Assay Kit kit is intended for research use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents of the b-HCG ELISA Assay Kit contain small amounts of Proclin 300<sup>R</sup> as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H<sub>2</sub>O<sub>2</sub> to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of β-HCG from 1 to 400 mIU/mL.

**5. PRECAUTIONS**

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the b-HCG ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all b-HCG ELISA Assay Kit kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange b-HCG ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested. To improve the performance of the kit on ELISA automatic systems, it is recommended to increase the number of washes.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond

ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate

- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

**6. PROCEDURE****6.1. Preparation of the Calibrators (C<sub>0</sub>...C<sub>5</sub>)**

The Calibrators are ready to use, are calibrated against the WHO 1<sup>st</sup> IRP 75/537 and have the following concentrations:

	C <sub>0</sub>	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
mIU/mL	0	1	5	20	100	400

The Calibrators are stable until the expiry date printed on the label. Once opened, the calibrators are stable six months at 2-8°C.

**6.2. Preparation of Wash Solution**

Dilute the content of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

**6.3. Preparation of Diluted Conjugate**

Prepare immediately before use.  
 Add 10 µL conjugate (reagent 4) to 1.0 mL of Incubation Buffer (reagent 3). The quantity of diluted Conjugate is proportional at the number of tests.  
 Mix gently for 10 minutes with a rotating mixer. Stable for 3 hours at room temperature (22-28°C).

**6.4. Preparation of the Sample**

β-HCG determination should be done in human serum or plasma.  
 Specimen can be stored at 2-8°C for at short time (max two days). For longer storage the specimen should be frozen at -20°C. Avoid repeated freezing and thawing.  
 For sample with concentration over 400 mIU/mL dilute the sample with Incubation buffer.  
 The Control is ready to use.

### 6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C<sub>0</sub>-C<sub>5</sub>), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/Control	Blank
Calibrator C <sub>0</sub> -C <sub>5</sub>	25 µL		
Sample/Control		25 µL	
Diluted Conjugate	100 µL	100 µL	
Incubate at room temperature (22±28°C) for 1 hour. Remove the contents from each well; wash the wells 3 times with 300 µL of diluted Wash Solution (if you use automated equipment, wash the wells at least 5 times). <b>Important note:</b> during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22±28°C) for 15 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

### 7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of β-HCG for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of

kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8. RESULTS

### 8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C<sub>0</sub>-C<sub>5</sub>) and of each sample.

### 8.2. Calibration curve

Plot the values of absorbance (Em) of the calibrators (C<sub>0</sub>-C<sub>5</sub>) against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic o Sigmoide).

### 8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in mIU/mL.

## 9. REFERENCE VALUES

Each laboratory must establish its own normal ranges based on subject population.

The serum β-HCG reference values are:

	Range (mIU/mL)
<b>Normal women</b>	< 8.0
<b>Pregnancy:</b>	
1° week	3.0 - 100
2° week	10 - 1000
3° week	100 - 10000
4° week	1000 - 100000
2° month	15000 - 200000
3° month	10000 - 100000

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

## 10. PERFORMANCE AND CHARACTERISTICS

### 10.1. Precision

#### 10.1.1. Intra Assay Variation

Within run variation was determined by replicate (16x) the measurement of three different control sera in one assay. The within assay variability is ≤ 7.6%.

#### 10.1.2. Inter Assay Variation

Between run variation was determined by replicate the measurement of three different control sera in different lots. The between assay variability is ≤ 8.8%.

### 10.2. Accuracy

The recovery of 6.25 - 12.5 - 25 - 50 mIU/mL of  $\beta$ -HCG added to sample gave an average value ( $\pm$ SD) of 99.2%  $\pm$  4.1% with reference to the original concentrations.

### 10.3. Specificity

Cross reactivity values of the  $\beta$ -HCG ELISA has been calculated on a weight/weight basis:

$\beta$ -HCG	100.0 %
hFSH	3.0 %
HCG	4.0 %
hTSH	0.02%

### 10.4. Sensitivity

The lowest detectable concentration of  $\beta$ -HCG that can be distinguished from the Calibrator 0 is 0.09 mIU/mL at the 95% confidence limit.

### 10.5. Correlation with RIA

Eagle Biosciences  $\beta$ -HCG ELISA (y) was compared to another commercially available  $\beta$ -HCG assay (x). Serum samples of 49 females were analysed according to both test systems.

The linear regression curve was calculated

$$y = 0.94 x - 0.02$$

$$r = 0.96 (r^2 = 0.92)$$

### 10.6. Hook Effect

$\beta$ -HCG ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 250.000 mIU/mL.

## 11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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## BIBLIOGRAPHY

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**DiaMetra S.r.l. Headquarter:** Via Garibaldi, 18 - 20090 SEGRATE (MI) Italy

Tel. +39-02-2139184

Fax +39-02-2133354.

**Manufactory:** Via Pozzuolo 14, 06083 SPELLO (PG) Italy

Tel. +39-0742-24851

Fax +39-0742-316197

E-mail:[info@diametra.com](mailto:info@diametra.com)

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distributed in the US/Canada by:

**EAGLE BIOSCIENCES, INC.**

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 FAX: 617-419-1110

[www.EagleBio.com](http://www.EagleBio.com) • [info@eaglebio.com](mailto:info@eaglebio.com)



## **ERROR POSSIBLE CAUSES / SUGGESTIONS**

### **No colorimetric reaction**

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

### **Too low reaction (too low ODs)**

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

### **Too high reaction (too high ODs)**

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

### **Unexplainable outliers**

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation