CE

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DCM014-13
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b-HCG ELISA

for routine analysis

Direct immunoenzymatic determination of β-HCG in human serum or plasma.

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO014

1. INTENDED PURPOSE

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

b-HCG ELISA is a manual *in vitro* diagnostic device intended for the quantitative determination of β-HCG in human serum or plasma from an adult population.

2. 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 syncytiotrophoblasts (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. Pregnancy tests employ an antibody that is specific to the β-subunit of HCG (β-HCG). This is important so that tests do not make false positives by confusing HCG with LH and FSH.

β-HCG is also secreted by some cancers including teratomas and choriocarcinomas. Elevated levels cannot prove the presence of a tumour and low levels do not rule it out.

3. PRINCIPLE OF THE METHOD

b-HCG ELISA is based on the simultaneous capture of HCG by a monoclonal antibody immobilised on the microplate and directed against the β-HCG fraction and another monoclonal antibody conjugated with peroxidase horseradish (HRP) and directed against the fraction β -HCG.

After the incubation, the bound/free separation is performed by a simple solid phase washing. Then, the enzyme HRP in the bound fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is proportional to the β-HCG concentration in the sample.

β-HCG concentration in the sample is calculated through a calibration curve.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

1. Calibrators (6 vials, 1 mL each)
ProClin >0.0015%, BSA 1.4%

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 indicated on the Certificate of Analysis. ProClin >0.0015%, BSA 1.4%
REF DCE045/1403-0

3. Incubation buffer (1 vial, 50 mL)
Phosphate buffer 50 mM pH 7.4, BSA 1 g/L. ProClin >0.0015%
REF DCE001/1401-0

4. Conjugate (1 vial, 1 mL)
Monocolonal anti- α-HCG subunit conjugated with horseradish peroxidase (HRP). ProClin >0.0015%, BSA 1.0%
REF DCE002/1402-0

5. Coated Microplate (1 microplate breakable)
Monocolonal anti- β-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)*
ProClin <0.0015%
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(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. Contains ProClin >0.0015%
REF DCE006-0

4.2. Materials required but not provided

Distilled water

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4.3. Auxiliary materials and instrumentation

Automatic dispenser
Precision Pipetting Devices
Microplate reader (450 nm, 620-630 nm)

5. WARNINGS

- This kit is intended for *in vitro* 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.
- ⚠ Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents (calibrators, control, incubation buffer, conjugate and wash solution) contain small amounts of ProClin™ 300 (>0.0015%, <0.06%) as preservative. Avoid contact with skin or mucosa.
- Classification according to Regulation (EC) No. 1272/2008 [CLP]

Skin sensitivity, Category 1

Warning

Contains: ProClin 300

Hazard statements:
H317 - May cause an allergic skin reaction.

Precautionary statements:
P261 - Avoid breathing dust / fume / gas / mist / vapours / spray.
P280 - Wear protective gloves/ protective clothing / eye protection / face protection / hearing protection.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.

- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous, corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to direct sunlight, metals or oxidants. Do not freeze the solution.

6. 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 should be stored refrigerated at 2 – 8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22 – 28°C) and mix well prior to use.
- Do not interchange 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 validated for its intended use/purpose.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- 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, icteric or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Fresh disposable tips must be used when pipetting assay reagents including samples, calibrators and controls to mitigate the risk of carryover contamination. Failure to do so may lead to invalid results.

7. REAGENT STORAGE AND STABILITY

Store the kit at 2 – 8°C in the dark.

- The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
- Once opened, the calibrators are stable at 2 – 8°C for 6 months*.
- The diluted wash solution is stable for 30 days at 2 – 8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

8. SAMPLE COLLECTION AND STORAGE

The assay should be performed using serum or plasma samples.

Sample Storage	Duration
2 – 8 °C	2 days
Freeze/thaw cycles	1 cycle

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9. PROCEDURE

9.1. Preparation of Calibrators and Controls

Before use, mix for 5 minutes with a rotating mixer.
The Calibrators are ready to use, are calibrated against the WHO 1st IRP 75/537 and have the following concentrations:

	C_0	C_1	C_2	C_3	C_4	C_5
mIU/mL	0	1	5	20	100	400

The control is ready to use; the concentration is printed on the label.

9.2. Preparation of the 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).

9.3. Preparation of the Wash Solution

Dilute the content of the vial "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.

It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 1000 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

9.4. Preparation of Samples

The determination of β-HCG can be performed in human serum or plasma samples.

Fasting samples are not necessary and no special sample preparations are required.

Collect blood by venepuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Store the sample at -20°C if the determination is not performed on the same day of the sample collection. Before using, mix gently, for 5 minutes, with a roller mixer.

9.5. Procedure

- **Allow all reagents to reach room temperature (22 – 28 °C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2 – 8°C: avoiding 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_0-C_5), two for the Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Calibrator C_0-C_5	**25 µL**		
Sample/ Control		**25 µL**	
Diluted conjugate	**100 µL**	**100 µL**	

Incubate 1 h at room temperature (22 – 28°C).
Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution.

Important note: 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.

Automatic washer: if you use automated equipment, wash the wells at least 5 times.

TMB Substrate	**100 µL**	**100 µL**	**100 µL**

Incubate at 22 – 28°C for 15 minutes in the dark.

Stop Solution	**100 µL**	**100 µL**	**100 µL**

Shake gently the microplate.
Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.

10. QUALITY CONTROL

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit controls provided in the kit should be tested as unknown and is intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of the control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.

DiaMetra recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

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When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[8].

11. CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit or Sigmoidal, **including Calibrator 0 is required.** Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

In order for the assay results to be considered valid the kit calibrators and control must fall within the specifications detailed in the lot specific certificate of analysis.

If a control is out of its specified range, the associated test results are invalid and samples must be retested.

NOTE: If the absorbance of a sample exceeds 400 mIU/mL, the sample should be diluted in **incubation buffer** and re-analysed.

12. EXPECTED VALUES

The serum β-HCG reference values are:

	Reference range (mIU/mL)
Non- pregnant	<8.0
Pregnancy	
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

The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

13. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

13.1. Precision

13.1.1. 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%.

13.1.2. 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%.

13.2. Accuracy

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

13.3. Specificity

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

β-HCG	100 %
hFSH	3.0 %
HCG	4.0 %
hTSH	0.02%

13.4. Sensitivity

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

13.5. Method comparison

b-HCG ELISA (y) was compared to another commercially available β-HCG assay (x). Serum samples of 49 females were analysed according to both test systems.
The linear regression curve was calculated:

n	**Slope**	**Intercept (mIU/mL)**	**Correlation coefficient (r)**
49	0.94	-0.02	0.96 (R^2 = 0.92)

13.6. Hook Effect

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

14. LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays[9]. Patients routinely exposed to animals or to animal serum products can be

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prone to this interference and anomalous values may be observed.

15. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

All materials that have come into contact with samples and reagents must be disposed of in accordance with country, state and local regulations.

16. BIBLIOGRAPHY

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4. Acosta, A.A.M.D. and Wright, G.L., Journal of Clinical Immunoassays, 6, 41 (1983).
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17. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

18. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.

The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

Ed. 03/2024 **DCM014-13**

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DiaMetra	Packaging Information Sheet	Mod. PIS000-2

IVD	DE	*In vitro* Diagnostikum		DE	Hergestellt von
	ES	Producto sanitario para diagnóstico *In vitro*		ES	Elaborado por
	FR	Dispositif medical de diagnostic *in vitro*		FR	Fabriqué par
	EN	*In vitro* Diagnostic Medical Device		EN	Manufacturer
	IT	Dispositivo medico-diagnostico *in vitro*		IT	Produttore
	PT	Dispositivos medicos de diagnostico *in vitro*		PT	Produzido por
	DE	Achtung, Begleitdokumente	yyyy-mm	DE	Herstellungs datum
	ES	Precaución, consulte los documentos adjuntos		ES	Fecha de fabricacion
	FR	Attention, veuillez consulter les documents d'accompagnement		FR	Date de fabrication
	EN	Caution, consult accompanying documents		EN	Date of manufacture
	IT	Attenzione, consultare la documentazione allegata		IT	Data di produzione
	PT	Atenção,consultar os documentos de acompanhamento		PT	Data de produção
yyyy-mm-dd	DE	Verwendbar bis		DE	Biogefährdung
	ES	Establa hasta (usar antes de último día del mes)		ES	Riesco biológico
	FR	Utiliser avant (dernier jour du mois indiqué)		FR	Risque biologique
	EN	Use by (last day of the month)		EN	Biological risk
	IT	Utilizzare prima del (ultimo giorno del mese)		IT	Rischio biologico
	PT	Utilizar (antes ultimo dia do mês)		PT	Risco biológico
	DE	Gebrauchsanweisung beachten	LOT	DE	Chargenbezeichnung
	ES	Consultar las instrucciones		ES	Codigo de lote
	FR	Consulter le mode d'emploi		FR	Numero de lot
	EN	Consult instructions for use		EN	Batch code
	IT	Consultare le istruzioni per l'uso		IT	Codice del lotto
	PT	Consultar instruções para uso		PT	Codigo do lote
Σ = xx	DE	Ausreichend für "n" Tests	CONT	DE	Inhalt
	ES	Contenido suficiente para "n" tests		ES	Contenido del estuche
	FR	Contenu suffisant pour "n" tests		FR	Contenu du coffret
	EN	Contains sufficient for "n" tests		EN	Contents of kit
	IT	Contenuto sufficiente per "n" saggi		IT	Contenuto del kit
	PT	Contém o suficiente para "n" testes		PT	Conteúdo do kit
	DE	Temperaturbereich	REF	DE	Bestellnummer
	ES	Límitaciôn de temperatura		ES	Nûmero de catálogo
	FR	Limites de température de conservation		FR	Réferéncès du catalogue
	EN	Temperature limitation		EN	Catalogue number
	IT	Limiti di temperatura		IT	Numero di Catalogo
	PT	Temperaturas limites de conservação		PT	Número do catálogo
	DE	Vor direkter sonneneinstrahlung schützen			
	ES	Mantener alejado de la luz solar			
	FR	Tenir à l'écart de la lumière du soleil			
	EN	Keep away from sunlight			
	IT	Tenere lontano dalla luce solare			
	PT	Mantenha longe da luz solar			

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