βhCG

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Beta-Human Chorionic Gonadotropin (ELISA)

REF: DS177712 Σ **96**

Intended use
The DiaSino βhCG assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of the sum of human chorionic gonadotropin (hCG) plus the hCG β-subunit in human serum. For professional use only.

Summary
References[1,2]
Similarly to LH (Luteinizing hormone), FSH (Follicle-stimulating hormone) and TSH (Thyroid-stimulating hormone), human chorionic gonadotropin (hCG) is a member of the glycoprotein family and consists of 2 subunits (α-1 and β-chains) which are associated to form the intact hormone. The α-chains in all four of these glycoprotein hormones are virtually identical, whereas the β-chains have greatly differing structures and are responsible for the respective specific hormonal functions. Specific assays for beta-hCG permit the early detection of pregnancy.

Test principle
Sandwich principle. Total duration of assay: **80 minutes.**
- Sample, monoclonal hCG-specific antibodies coated microwells and enzyme labeled monoclonal hCG-specific antibodies are combined.
- During the incubation, β-hCG presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the β-hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the β-hCG within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of β-hCG in the sample.

Reagents
Materials provided
- **βhCG Coated Microplate - symbol** βhCG PLATE 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal hCG-specific antibodies.
- **βhCG Calibrators - symbols** βhCG CAL A-F 6 vials, 1 mL each, ready to use; Concentrations: 0(**A**), 5(**B**), 20(**C**), 50(**D**), 100(**E**) and 200(**F**) mIU/mL.
- **βhCG Enzyme Conjugate - symbol** βhCG CONJ 1 vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal hCG-specific antibodies in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- **βhCG Sample Diluent Concentrate - symbol** βhCG DILUT 1 vial, 11 mL of serum diluent concentrate containing buffer containing 0.1% ProClin300 preservative.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store at 2-8°C.
- Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Calibration
The DiaSino βhCG ELISA has been standardized against the 4th International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (NIBSC) code 75/551.
Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Sample Dilute (10X dilution)
Add deionized water to the 10X Sample Diluent Concentrate.
Dilute 11 mL of Sample Diluent Concentrate to 99 mL of deionized water to a final volume of 110 mL. Stable for 2 months at 2-8°C.

Test procedure
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add **25 µL** of **calibrators** or **samples** to each well.
- Add **100 µL** of **enzyme conjugate** to each well.
- Shake the **microplate** gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37 °C** for **60 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **substrate** to each well.
- Cover and **Incubate** at **ambient temperature (18-25°C)** in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
- Add **50 µl** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in mIU/mL for each calibrator.

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- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (mIU/mL)	Absorbance
Calibrator A	0	0.017
Calibrator B	5	0.124
Calibrator C	20	0.352
Calibrator D	50	0.686
Calibrator E	100	1.539
Calibrator F	200	2.811
Control 1	24.3	0.4
Control 2	139.2	2.04
Sample	75.8	1.13

Limitations - interference
- The assay is unaffected by icterus (bilirubin < 410 µmol/L or < 24 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1400 mg/dL) and biotin (< 327 nmol/L or < 80 ng/mL).
- Criterion: Recovery within ± 10 % of initial value.
- No interference was observed from rheumatoid factors up to a concentration of 3400 IU/mL and samples from dialysis patients.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
0.500-200 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL (or up to 20000 mIU/mL for 100-fold diluted samples).

Lower detection limit
0.500 mIU/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n=21).

Expected values
Results from a multicenter study in clinical centers are listed below.
- **≤ 3 mIU/mL** hCG for 97.5 % of the values obtained from 286 healthy, non-pregnant premenopausal women. The corresponding upper 95 % confidence limit ranges up to **5.5 mIU/mL**.
- **≤ 7 mIU/mL** hCG for 97.5 % of the values obtained from 133 healthy, postmenopausal women. The corresponding upper 95 % confidence limit ranges up to **8.5 mIU/mL**.
- **< 2.5 mIU/mL** hCG for 97.5 % of the values obtained from 369 men. The corresponding upper 95 % confidence limit ranges up to **3.0 mIU/mL**.
- During pregnancy (weeks of pregnancy - defined as completed weeks of pregnancy beginning with the start of the last menstruation phase), the following values have been determined.

Data are given only for the weeks of gestation for which the case numbers (n) were greater than 10.

Weeks of gestation	N	hCG mIU/mL	
		Median	5-95th percentile
3	17	16.8	6.6-58.3
4	57	158	11.4-692
5	41	1477	198-8009
6	39	3899	265->20000
7	25	>20000	3787->20000
8-12	17	>20000	>20000

Median values and the 5th and 95th percentile were calculated for the completed gestational weeks - see within the table above.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

Sample		Repeatability*		Intermediate precision	
	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human Serum 1	3.58	0.22	6.13	0.31	8.79
Human Serum 2	37.11	2.02	5.44	2.33	6.27
Human Serum 3	156.94	6.72	4.28	9.59	6.11
PC Universal 1	52.78	3.08	5.84	3.53	6.69
PC Universal 2	133.63	5.51	4.12	7.66	5.73

*Repeatability = within-run precision

Method comparison
A comparison of the DiaSino βhCG assay (y) with the Elecsys HCG+β (x) using 121 clinical samples gave the following correlations:

Linear regression
y = 1.036x - 1.095
r = 0.9882

The sample concentrations were between approx. 1 and 18550 mIU/mL.

Analytical specificity
For the monoclonal antibodies used, the following cross-reactivities were found:
TSH not detectable, LH 0.12 %, FSH < 0.1 %.

Functional sensitivity
0.510 mIU/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Hook effect
There is no high-dose hook effect at hCG concentrations up to 1000000 mIU/mL.

References

1. Schwarz S, Berger P, Wick G. The Antigenic Surface of Human Chorionic Gonadotropin as Mapped by Murine Monoclonal Antibodies. Endocrinology 1986;118(1):189-197.
2. Sturgeon CM, McAllister EJ. Analysis of hCG: clinical applications and assay requirements. Ann Clin Biochem 1998;35:460-491.

Symbols

IVD In vitro diagnostic medical device	8°C / 2°C Temperature limit	Consult instructions for use	REF Catalog number
LOT Batch code	Date of manufacture	Use-by date	Σ Contains sufficient for <n> tests
Manufacturer	Do not use if package is damaged and consult instructions for use	CE European Conformity	EC REP Authorized representative in the European Community

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