Free Beta Human Chorionic Gonadotropin (β-HCG) ELISA

For the quantitative determination of Free β-HCG in human serum and EDTA plasma.

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 20-FBEHU-E01
Size: 96 wells
Version: 12.1 2024/02 - ALPCO 1.1

1. Intended Use

The Free Beta Human Chorionic Gonadotropin (β-HCG) ELISA is an enzyme immunoassay for the quantitative determination of the free beta subunit of human chorionic gonadotropin (free β-hCG) in human serum and EDTA plasma. For research use only. Not for use in diagnostic procedures.

2. Principle of the Assay

The Free β-hCG ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the sandwich principle. The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on a free β-hCG molecule. An aliquot of sample containing endogenous free β-hCG is incubated in the coated well with enzyme conjugate, which is an anti- β-hCG antibody (rabbit) conjugated with horseradish peroxidase. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of free β-hCG in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of free β-hCG in the sample.The colorimetric reaction is stopped by addition of stop solution, and the optical density (OD) of the resulting yellow product is measured. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3. Warnings and Precautions

1. This kit is for research use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA-approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn the solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21°C to 26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.

12. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national and local biohazard safety guidelines or regulations.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may be slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND, and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with plenty of water and skin with soap and plenty of water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents must be treated as hazardous waste according to the local and national biohazard safety guidelines or regulations.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets.

4. Reagents

4.1 Reagents Provided

Component	Quantity	Description	Preparation
Microplate wells	12 x 8 wells (break apart)	**Microplate** Coated with anti-β-HCG antibody (monoclonal).	Ready to use
***Standard** **(Standard 0 - 5)**	6 x 1 mL	**Standards** Concentrations: 0 – 10 – 25 – 50 – 100 – 200 ng/mL; Conversion: 1 mIU = 1 ng The concentrations of the Free β-HCG Kit standards match the WHO Reference Reagent Human Chorionic Gonadotrophin, Beta Subunit (Purified) (NIBSC code: 99/650)	Lyophilized See "Reagent Preparation"
***Control (Low and High)**	2 x 1 mL	**Control (Low and High)** For control values and ranges please refer to vial label or QC-Datasheet.	Lyophilized See "Reagent Preparation"
***Zero Buffer**	1 x 14 mL	**Zero Buffer** .	Ready to Use
Enzyme Conjugate	1 x 18 mL	**Enzyme Conjugate** Anti β-HCG antibody conjugated to horseradish peroxidase.	Ready to use
Substrate Solution	1 x 14 mL	**Substrate Solution** Tetramethylbenzidine (TMB). *Keep away from light.*	Ready to use
Stop Solution	1 x 14 mL	**Stop Solution** contains 0.5M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.	Ready to use

Wash Solution	1 x 30 mL	**Wash Solution** 40x concentrated	40x concentrated See “Reagent Preparation”

*Contains non-mercury preservative.

4.2 Materials Required but Not Provided

- A calibrated microtiter plate reader (450 nm ± 10 nm)
- Calibrated variable precision micropipettes
- An incubator suitable for incubation at 37°C
- Absorbent paper
- Distilled or deionized water
- Timer (60 min. range)
- Linear graph paper or software for data reduction

4.3 Storage Conditions

Unopened kits and reagents as well as opened reagents must be stored at 2°C to 8°C. When stored at 2°C - 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened kits retain activity for four weeks if stored as described above and within the expiration date on the kit label.

The microtiter plate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch including the desiccant and used in the plate frame provided. Once the foil bag has been opened, care must be taken to close it tightly again. Once opened, reagent vials must be closed tightly again.

	Storage Temperature	Stability
Unopened kits and unopened reagents	2°C to 8°C.	Until expiration date printed on the label. Do not use reagents beyond this date!
Opened Kit	2°C to 8°C.	Four weeks if within printed expiration date

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (21°C to 26°C) prior to use.

Standards

Reconstitute the lyophilized contents of the standard vials with 1.0 mL distilled water and let stand for 10 minutes at minimum. Mix several times before use.

Note: *The reconstituted standards are stable for up to 30 days at 2°C - 8°C.*
For longer storage freeze at -20°C.

Controls

Reconstitute the lyophilized content of the control vials with 1.0 mL distilled water and let stand for 10 minutes at minimum. Mix several times before use.

Note: *The reconstituted controls are stable for up to 30 days at 2°C - 8°C.*
For longer storage freeze at -20°C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
Note: *The 1X working Wash Solution is stable for 1 week at room temperature.*

4.5 Kit Disposal
The disposal of the kit must be according to local and national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits
In case of any severe damage to the test kit or components, ALPCO must be informed in writing, no later than one week after receiving the kit. Severely damaged single components should not be used for a test run. They must be stored until a final resolution has been found. After this, they should be disposed according to official regulations.

5. Sample Collection and Preparation
Serum or EDTA plasma should be used in this assay.
Do not use hemolytic, icteric, or lipemic samples.
Note*:* Samples containing sodium azide should not be used in the assay.

5.1 Sample Collection
Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.
EDTA plasma: Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Sample Storage and Preparation
Samples should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Samples held for a longer time (up to 12 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution
If in an initial assay, a sample is found to contain more than the highest standard, the samples can be diluted with *Zero Buffer* and re-assayed as described in the Assay Procedure.
For calculating concentrations, this dilution factor must be considered.
Example:
a) dilution 1:10: 10 µL sample + 90 µL *Zero Buffer* (mix thoroughly)
b) dilution 1:100: 10 µL 1:10 dilution (a) + 90 µL *Zero Buffer* (mix thoroughly)

6. Assay Procedure

6.1 General Remarks
- All reagents and samples must be allowed to come to room temperature before use.
- All reagents must be mixed without foaming.
- Use new disposable plastic pipette tips for each standard, control, or sample to avoid cross-contamination.
- The enzymatic reaction is linearly proportional to time and temperature.
- Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

- Do not interchange caps of reagent vials to avoid cross-contamination.
- Once the test has been started, all steps should be completed without interruption.

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of microplate wells in the holder.
2. Dispense **50 µL** of each ***Standard, Control,*** and **sample** with new disposable tips into appropriate wells.
3. Dispense **100 µL *Zero Buffer*** into each well.
   Thoroughly mix for 30 seconds. It is important to completely mix in this step.
4. Incubate for **30 minutes** at 37°C.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with 1X working Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense **150 µL *Enzyme Conjugate*** into each well.
7. Incubate for **30 minutes** at 37°C.
8. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with 1X working Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **100 µL** of ***Substrate Solution*** to each well.
10. Incubate for **20 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **100 µL** of **Stop Solution** to each well.
    It is important to make sure that all the blue color changes to yellow color completely.
12. Determine the absorbance (OD) of each well at **450 ±10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 15 minutes** after adding the *Stop Solution*.

6.3 Calculations of Results

1. Calculate the average absorbance values for each set of standards, controls, and samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Paramer Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard must be further diluted or reported as such. For the calculation of the concentrations this dilution factor must be considered.

6.3.1 Example of Typical Standard Curve

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

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	0.02
Standard 1 (10 ng/mL)	0.22
Standard 2 (25 ng/mL)	0.46
Standard 3 (50 ng/mL)	0.81
Standard 4 (100 ng/mL)	1.28
Standard 5 (200 ng/mL)	1.97

7. Expected Normal Values

It is strongly recommended that each laboratory determine its own normal and abnormal values.

8. Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

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

The controls and the corresponding results of the QC Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs to ensure the accuracy of the results.

Employ appropriate statistical methods to analyze control values and trends. If the results of the assay do not agree with the established acceptable ranges of control materials, results should be considered invalid. In this case, please check the following technical areas: pipetting and timing devices, photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above-mentioned items without finding any error, please contact ALPCO.

9. Performance Characteristics

9.1 Assay Dynamic Range

The range of the assay is between 0.2 – 200 ng/mL.

9.1 Assay Sensitivity

Limit of Blank (LoB)	0.06 ng/mL
Limit of Detection (LoD)	0.22 ng/mL
Limit of Quantitation (LoQ)	1.00 ng/mL

9.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross reactivity of the assay:

Substance	Concentration (µIU/mL)	Found concentration (ng/mL)
TSH	25	< 0.3 ng/mL
FSH	100	< 0.2 ng/mL
Prolactin	100	< 0.5 ng/mL
LH	200	< 1 ng/mL

Substance	Concentration (µIU/mL)	Mean Cross-reactivity (%)
TSH	100	3.1
FSH	50	2.3
Prolactin	50	3.2
LH	100	-4.3

10. Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Hemoglobin (up to 4 mg/mL), Bilirubin (up to 0.125 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Currently no substances (drugs) are known for this assay, which have an influence on the measurement of free β-HCG in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 19800 ng/mL of free β-HCG.

11. Legal Aspects

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. If there is any doubt or concern regarding a result, please contact ALPCO.

11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results are also invalid. In the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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