Human Chorionic Gonadotropin (HCG) ELISA

For the quantitative determination of intact hCG in human serum and plasma (EDTA, lithium heparin, or citrate).

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

Catalog Number: 20-HCGHU-E01
Size: 96 wells
Version: 11.1 2023/09 - ALPCO 2.1

1. Intended Use

The ALPCO HCG ELISA is an enzyme immunoassay for the quantitative determination of intact human chorionic gonadotropin (hCG) in serum or plasma (EDTA, lithium heparin, or citrate plasma). For research use only. Not for use in diagnostic procedures.

2. Principle of the Assay

The HCG ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the sandwich principle. The microplate wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on an hCG molecule. An aliquot of sample containing endogenous hCG is incubated in the coated well with enzyme conjugate, which is a monoclonal antibody directed against the alpha-chain of hCG conjugated with horseradish peroxidase. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of hCG in the sample. After addition of the substrate solution, the intensity of color developed is proportional to the concentration of hCG in the sample. 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 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 biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit label.

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 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.
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 Sheet available upon request from ALPCO.

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 (Standards 1 - 5)**	5 x 1 mL	**Standards** Concentrations: 5; 50; 200; 500; 1000 mIU/mL Conversion: 1 pg/mL = 0.00916 mIU/mL *The standards are calibrated against the following reference material: 5th WHO International Standard Chorionic Gonadotrophin NIBSC code: 07/364*	Lyophilized See "Reagent Preparation"
***Sample Diluent**	1 x 10 mL	**Sample Diluent**	Ready to Use
***Enzyme Conjugate**	1 x 11 mL	**Enzyme Conjugate** Monoclonal antibody against the alpha-subunit 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

*Contains non-mercury preservative.

4.2 Materials Required but Not Provided

- A calibrated microplate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes

- Absorbent paper
- Distilled water
- Timer
- Linear graph paper or software for data reduction
- Centrifuge (for sample preparation)

4.3 Storage Conditions

Unopened kits and reagents as well as opened reagents must be stored at 2 °C - 8 °C. When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

The microplate contains snap-off strips. Unused wells must be stored at 2 °C - 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.

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 mL distilled water and let stand for at least 10 minutes. Mix several times before use.

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

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 of according to official regulations.

5. Sample Collection and Preparation

Serum or plasma (EDTA, lithium heparin, or citrate 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.
For further information refer to *Interfering Substances*.

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.

Plasma: Whole blood should be collected into centrifuge tubes containing 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 5 days at 2 °C to 8 °C prior to assaying. Samples held for a longer time 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 hCG than the highest standard, the samples can be diluted with *Sample Diluent* 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 *Sample Diluent* (mix thoroughly)
b) dilution 1:100: 10 µL of 1:10 dilution (a) + 90 µL *Sample Diluent* (mix thoroughly).

Note: Sera samples collected during pregnancy must be diluted 1:100 in *Sample Diluent* before starting the assay.

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

6.2 Assay Procedure (Quantitative Method)

Each run must include a standard curve.
NOTE: Sera samples collected during pregnancy must be diluted 1:100 in *Sample Diluent* before starting the assay (see 5.3.)

1. Secure the desired number of microplate wells in the holder.
2. Dispense **25 µL** of each ***Standard, Control,*** and **sample** with new disposable tips into appropriate wells.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to completely mix in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with distilled water (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 correct washing!
6. Add **100 µL** of ***Substrate Solution*** to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
9. Measure the optical density (OD) of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microplate reader. Read **within 10 minutes** after adding the *Stop Solution*.

6.3 Assay Procedure (Qualitative Method)

This procedure differentiates positive (pregnant) from negative samples by comparing the sample hCG levels with *Standard 1* (5 mIU/mL) and *Standard 2* (50 mIU/mL).
Samples are run in parallel with the *Standard 1* (5 mIU/mL) and the 50 mIU/mL *Standard 2*. The assay procedure is identical to the Quantitative Method, **but Steps 8 and 9 are omitted**.

6.4 Calculations of Results

1. Calculate the average absorbance (OD) values for each set of standards, controls, and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample, determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit (4 Parameter Rodbard or 4 Parameter 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 > 1000 mIU/mL. For the calculation of the concentrations this dilution factor must be considered.

6.4.1 Example of Typical Standard Curve

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

Standard	Optical Units (450 nm)
Standard 1 (5 mIU/mL)	0.05
Standard 2 (50 mIU/mL)	0.14
Standard 3 (200 mIU/mL)	0.43
Standard 4 (500 mIU/mL)	0.94
Standard 5 (1000 mIU/mL)	1.54

7. Qualitative Results

For qualitative analysis of the hCG level, the color development of the sample is compared with the color of *Standard 1 (*5 mIU/mL) and *Standard 2* (50 mIU/mL).
If the blue color is less intense than the color of the 50 mIU/mL Standard, the sample is considered negative.
If the blue color is more intense than or equal to the color of the 50 mIU/mL Standard the sample is considered positive.

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 5 – 1000 mIU/mL.

9.2 Specificity of Antibodies (Cross-Reactivity)

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

Protein	Concentration	Produced Color Intensity Equivalent to HCG in serum (mIU/mL)
hLH	300 mIU/mL	9
hLH	200 mIU/mL	< 5
hLH	80 mIU/mL	< 5
TSH	75 µIU/mL	10
TSH	50 µIU/mL	6
TSH	25 µIU/mL	< 5
FSH	200 mIU/mL	< 5
FSH	50 mIU/mL	< 5

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 0.5 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 to influence the measurement of hCG in a sample in this assay.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 13,300 mIU/mL of hCG.

11. Legal Aspects

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. 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, enough 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.