Human Placental Lactogen / hPL ELISA Kit

Enzyme Immunoassay for the quantification of Human Placental Lactogen / hPL in serum.

Catalog number: ARG80846

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INTRODUCTION

Human placental lactogen (hPL), also called human chorionic somatomammotropin (HCS), is a polypeptide placental hormone, the human form of placental lactogen (chorionic somatomammotropin). Its structure and function are similar to those of human growth hormone. It modifies the metabolic state of the mother during pregnancy to facilitate the energy supply of the fetus. hPL has anti-insulin properties. hPL is a hormone secreted by the syncytiotrophoblast during pregnancy. Like human growth hormone, hPL is encoded by genes on chromosome 17q22-24. It was identified in 1963.

hPL is present only during pregnancy, with maternal serum levels rising in relation to the growth of the fetus and placenta. Maximum levels are reached near term, typically to 5–7 mg/L (5-7 micrograms/ml). Higher levels are noted in patients with multiple gestation. Little hPL enters the fetal circulation. Its biological half-life is 15 minutes. [Provide by Wikipedia:Human placental lactogen (hPL)]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for hPL has been pre-coated onto a microtiter plate. Standards, Controls or samples are pipetted into the wells and any hPL present is bound by the immobilized antibody. After washing away any unbound substances, an antibody-conjugate specific for hPL is added to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of hPL bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm. The concentration of hPL in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for six weeks if stored as described.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standards (1.25, 5, 10, 20 mg/L)	0.5 mL each (ready to use)	4°C
Control (Low)	2 vials, 0.5 mL each (ready to use)	4°C
Control (High)	2 vials, 0.5 mL each (ready to use)	4°C
Diluent Buffer (dyed red)	90 mL	4°C
Antibody Conjugate (Anti-hPL Antibody-Peroxidase)	11 mL (ready to use)	4°C
TMB substrate	14 mL (ready to use)	4°C (protect from light)
STOP solution	14 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents to room temperature (21-26°C).
- For the calculation of the concentrations this dilution factor has to be taken into account.
- Manual Washing: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes. Each run must include a standard curve.
- Automated Washing: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes. Each run must include a

standard curve.

- Briefly spin down the all vials before use.
- If crystals are observed in the Diluent Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. Before starting the assay the sample must be pre-diluted 1:100 with Diluent Buffer and mix for 10 seconds on a Vortex mixer (avoid foaming).

Note:

1. The internal controls, Control (Low) & (High) are ready to use and Do not be diluted.
2. If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be further diluted with Diluent Buffer.
3. Do not use haemolytic, icteric or lipaemic specimens.
4. Samples containing sodium azide should not be used in the assay.
5. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in 1X Diluent Buffer as necessary.

REAGENT PREPARATION

Bring all reagents and required number of strips to room temperature prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards and samples should be assayed in duplicates.

1. Add **10 µL** of **prediluted samples, Control (Low) & (High) and Standards** to the Antibody-coated microplate.
2. Add **100 µL** of the **Antibody Conjugate** to each well. Thoroughly mix for **10 seconds.** It is important to have a complete mixing in this step.
3. Incubate at **RT** for **30 mins.**
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with **distilled water** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
5. Warm **TMB substrate** to **RT**. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **10 minutes** at room temperature in dark.
6. Add **50 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 60 ppm standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Standard	Absorbance (450 nm)
Diluent Buffer (0 mg/L)	0.03
Standard (1.25 mg/L)	0.17
Standard (5 mg/L)	0.65
Standard (10 mg/L)	1.17
Standard (20 mg/L)	1.84

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. Using log-log, semi-log or linear 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. Use 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 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above. dta

QUALITY ASSURANCE

Sensitivity

0.043 mg/L

Intra-assay and Inter-assay precision

The CV value of intra-assay was 5.55-6.73% and inter-assay precision was 5.67-8.82%

Recovery

Samples have been spiked by adding hPL solutions with known concentrations to three different sera containing different amounts of endogenous analytic. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100. (Data shown in following page)

Sample	Endogenous hPL (mg/L)	Added Conc. (mg/L)	Measured Conc. (mg/L)	Expected Conc. (mg/L)	Recovery (%)
1	0.00	0.00	0.00		
		0.63	0.55	0.63	88.60
		2.50	2.40	2.50	96.20
		5.00	5.21	5.00	104.20
		10.00	8.58	10.00	85.80
2	1.93	0.00	1.93		
		0.63	1.39	1.59	87.60
		2.50	3.16	3.46	91.40
		5.00	5.21	5.96	87.40
		10.00	9.81	10.96	89.50
3	4.67	0.00	4.67		
		0.63	2.56	2.96	86.40
		2.50	4.46	4.83	92.30
		5.00	6.69	7.33	91.20
		10.00	11.66	12.33	94.50

Cross Reactivity

The following substances were tested for cross reactivity of the assay

Antigen tested	Equivalent to hPL
hCG 2000 IU/L	undetectable
AFP 300 KIU/L	undetectable
hGH 100 µg/L	undetectable
Prolactin 200 µg/L	undetectable