hPL ELISA

KAPD1283

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History

Summary of change :

Previous Version : 200224/1	**Current Version :** 230718
Old DiaSource logo	New DiaSource logo on the front page

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IN VITRO DIAGNOSTIC USE

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1 INTRODUCTION

The DIAsource hPL Enzyme Immunoassay Kit provides materials for the quantitative determination of human Placental Lactogen (hPL) in serum. This assay is intended for in vitro diagnostic use only.
The physiological role of HPL has not yet been established, but the great similarity to human growth hormone has stimulated the hypothesis about a function as a regulator of feto-placental growth and other physiological alterations during pregnancy. It has been suggested that the maternal serum-level may reflect on "index of placental function".
Depressed levels of HPL are seen in association with intrauterine death, fetal distress in labor and birth asphyxia. This association is particularly strong if depressed levels are seen repeatedly, implying a chronic state of placental and therefore fetal compromise. Depressed levels are usually not present if the pregnancy has proceeded uneventfully to term.
Elevated levels of HPL are usually indicative of optimal pregnancy outcome in singleton pregnancies. However, high levels may indicate substantial fetal pathology in specific diseases, namely diabetes mellitus and fetal macrosomia, rhesus isoimmunization and hydrops fetalis.

2 PRINCIPLE OF THE TEST

The DIAsource hPL 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 an hPL molecule. An aliquot of patient sample containing endogenous hPL is incubated in the coated well with enzyme conjugate, which is a monoclonal anti-hPL antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of hPL in the sample.
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of hPL in the patient sample.

3 PRECAUTIONS

1. This kit is for in vitro diagnostic 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 the package insert 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 patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens 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 labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate 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 result 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 have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
    Material Safety Data Sheets for this product are available upon request directly from DIAsource.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. 12 x 8 (break apart) strips, 96 wells
   Wells coated with anti-hPL antibody (monoclonal)

2. CAL | N
   4 vials, 0.5 ml, ready to use
   Concentrations: 1.25 – 5.0 – 10.0 – 20 mg/L
   Conversion: 1 mg/L = 1 mIU/L
   The calibrators are calibrated against the NIBSC International Standard for hPL IRP (73/545)
   contain non-mercury preservative.

3. CAL | 0
   Zero Calibrator (also used as Sample Diluent), 1 vial, 90 mL, ready to use
   0 mg/L
   contain non-mercury preservative.

4. CONTROL | N
   2 vials, 0.5 mL each, ready to use
   For control values and ranges please refer to vial label or QC-Datasheet.
   Contain non-mercury preservative.

5. Ab | HRP
   Enzyme Conjugate, 1 vial, 11 mL, ready to use
   Anti-hPL antibody conjugated to horseradish peroxidase
   contain non-mercury preservative.

6. CHROM | TMB
   Substrate Solution, 1 vial, 14 mL, ready to use
   Tetramethylbenzidine (TMB)

7. STOP | SOLN
   Stop Solution, 1 vial, 14 mL, ready to use
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

Note: Additional *Zero Calibrator* for sample dilution is available on request.

4.2 Equipment and material required but not provided

- A microtiter plate calibrated reader (450±10 nm).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.
- Tubes for sample dilution (12x75 mm)

4.3 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. 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 above.

4.4 Preparation of Reagents

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

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, DIAsource have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum must be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen 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. Patients receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen storage and preparation

Specimens should be capped and may be stored for up to 5 days at 2°C to 8°C prior to assaying.
Specimens 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 Specimen dilution

Before starting the assay the sample must be **prediluted 1:100 with Zero Calibrator**:

1. Add for each sample 1mL of Zero Calibrator in one test tube.
2. Add 10µL of each sample to the appropriate test tube. Mix all tubes for 10 seconds on a Vortex mixer (avoid foaming).

The internal controls (Control Low & High) are ready to use and need not to be diluted.
External controls (such as Bio-Rad controls) has to be handled like samples.

If in an initial assay, a specimen is found to contain more than the highest calibrator, the specimens can be further diluted with *Zero Calibrator* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each calibrator, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation 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.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Assay Procedure

NOTE:
Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all calibrators, samples, and controls should be completed within 3 minutes.
Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all calibrators, samples, and controls be completed within 3 minutes.
Each run must include a calibration curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **10 µL** of each ***Standard,*** **control** and **prediluted samples** 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 have a complete mixing 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 (300 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. 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. Read the OD at **450±10 nm** with a microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a calibration curve by plotting the mean absorbance obtained from each calibrator 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 calibration 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 calibration curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted or reported as > 20mg/L. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 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.

Calibrator	Optical Units (450 nm)
Zero Calibrator (0 mg/L)	0.03
Calibrator 1 (1.25 mg/L)	0.17
Calibrator 2 (5.0 mg/L)	0.65
Calibrator 3 (10.0 mg/L)	1.17
Calibrator 4 (20.0 mg/L)	1.84

7 EXPECTED VALUES

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

In a study, using the DIAsource hPL ELISA, the following values are observed:

Pregnancy week	Value [mg/L]
10. - 12.	0.05 - 1.00
12. - 14.	0.10 - 1.7
14. - 16.	0.3 - 2.8
16. - 18.	0.5 - 3.5
18. - 20.	0.9 - 4.0
20. - 22.	1.1 - 5.0
22. - 24.	1.3 - 5.8
24. - 26.	1.6 - 6.7
26. - 28	2.0 - 7.7
28. - 30.	2.7 - 8.5
30. - 32.	3.2 - 9.5
32. - 34.	3.7 - 10.1
34. - 36.	4.0 - 10.7
36. - 38.	4.3 - 11.2
38. - 40	4.4 - 11.7
40. - 42.	4.3 - 11.6

* Example:
If a patient is in the first half of pregnancy week 12 please use the range for weeks 10 - 12. If the patient is in the second half of week 12 then the range for weeks 12 - 14 should be used.
The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

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. Use controls at both normal and pathological levels.
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 in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient 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 contact your distributor or DIAsource directly.

9 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range
The range of the assay is between 0.04 – 20 mg/L.

9.2 Specificity of Antibodies (Cross Reactivity)
The following substances were tested for cross reactivity of the assay:

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

9.3 Analytical Sensitivity
The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of the Zero *Calibrator* and was found to be 0.043 mg/L.

9.4 Precision

9.4.1 Intra Assay Variation
The within assay variability is shown below:

Sample	n	Mean (mg/L)	CV (%)
1	18	0.66	6.06
2	18	2.34	5.55
3	18	6.24	6.73

9.4.2 Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (mg/L)	CV (%)
1	39	0.68	8.82
2	24	2.52	7.14
3	24	6.87	5.67

9.5 Recovery

Samples have been spiked by adding hPL solutions with known concentrations to three different sera containing different amounts of endogenous analyt. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100..

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

9.6 Linearity

Sample	Dilution	Measured Conc. (mg/L)	Expected Conc. (mg/L)	Recovery (%)
1	None	1.93	1.93	
	1:2	0.84	0.96	86.7
	1:4	0.45	0.48	93.7
	1:8	0.26	0.24	108.0
	1:16	0.12	0.12	95.5
2	None	4.67	4.67	
	1:2	2.28	2.33	97.5
	1:4	1.06	1.17	90.7
	1:8	0.64	0.58	109.2
	1:16	0.31	0.29	106.3

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

Haemoglobin (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

Until today no substances (drugs) are known to us, which have an influence to the measurement of hPL in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 700 mg/L of hPL.

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. In case of any doubt or concern please contact DIAsource.

11.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutical consequences.

11.3 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 subject to point 11.2. are also invalid. Regardless, 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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