



hPL Elisa

BL-19-E
IN VITRO DIAGNOSTIC USE



Bio-Line s.a. - Rue André Fauchille, 17- B1150 Brussels, Belgium - Tel: +32 2 736 62 18 - Fax : +32 2 742 13 15

1 CLINICAL RELEVANCE

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

Bio-Line hPL ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a hPL molecule. An aliquot of patient sample containing endogenous hPL is incubated in the coated well with enzyme conjugate, which is an anti-hPL antiserum conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off with water. 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 serum.

3 REAGENTS

3.1 Contents of the Kit

- | |
|--|
| |
|--|

 12x8 (break apart) strips, 96 wells
Wells coated with anti-hPL monoclonal antibody
- | | |
|-----|---|
| CAL | N |
|-----|---|

 N=1 to 4, Reference Calibrator Set, 4 vials, 0.5 ml each
See exact values on vial labels
- | | |
|-----|---|
| CAL | 0 |
|-----|---|

 Specimen Diluent
1 vial, 90 ml
- | | |
|----|-----|
| Ab | HRP |
|----|-----|

 Enzyme-Conjugate, 11 ml
Anti-hPL antiserum conjugated to horseradish peroxidase
- | | |
|-------|-----|
| CHROM | TMB |
|-------|-----|

 1 vial, 11 ml
Substrate Solution TMB
- | | |
|------|------|
| STOP | SOLN |
|------|------|

 1 vial, 6 ml
0.5M H₂SO₄

3.2 Equipment and material required but not provided

- A microtiterplate reader (450±10 nm).
- Precision micropipettes with disposable tips for 10 µl, 50µl, 100 µl and 1000 µl.
- Standard refrigerator.
- Mixing tubes (1 per sample) (uncoated standard tubes 12 x 75 mm).
- Vortex mixer or equivalent.
- Absorbent paper.

3.3 Storage conditions

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme Conjugate, Substrate Solution, Calibrators and Zero Calibrator* must be stored at 2° to 8°C.
- Microtiter wells* must be stored at 2° to 8°C. Once the foilbag has been open care should be taken to close it tightly again. The immunoreactivity of the coated microtiter wells is stable for approx. 6 weeks in the broken, but tightly closed bag containing the desiccant.

3.4 Normal Values

Pregnancy Diagnosis	Singleton Pregnancy		Twin Pregnancy	
10 - 12 Weeks	0.05	-1.00		
12 - 14 Weeks	0.10	-1.7		
14 - 16 Weeks	0.3	-2.8		
16 - 18 Weeks	0.5	-3.5		
18 - 20 Weeks	0.9	-4.0		
20 - 22 Weeks	1.1	-5.0	2.2	- 7.0
22 - 24 Weeks	1.3	-5.8	3.0	- 8.1
24 - 26 Weeks	1.6	-6.7	3.6	- 9.2
26 - 28 Weeks	2.0	-7.7	4.1	-10.3
28 - 30 Weeks	2.7	-8.5	4.6	-11.7
30 - 32 Weeks	3.2	-9.5	5.0	-13.0
32 - 34 Weeks	3.7	-10.1	5.4	-15.1
34 - 36 Weeks	4.0	-10.7	5.7	-17.8
36 - 38 Weeks	4.3	-11.2	5.9	-19.7
38 - 40 Weeks	4.4	-11.7	5.8	-19.6
40 - 42 Weeks	4.3	-11.6	--	- --

3.5 Warnings and precautions for users

- CAUTION:** Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).
- Avoid contact with *Stop Solution* 0.5M H₂SO₄. It may cause skin irritation and burns.
- Replace caps on reagents immediately. Do not switch caps.
- Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
- Do not pipette reagents by mouth.
- For in vitro diagnostic use only.
- Do not mix or use components from kits with different lot numbers.

4 SPECIMEN COLLECTION AND PREPARATION

- Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Avoid hemolysis. This kit is for use with serum samples without additives only.
- Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimen 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 PERFORMANCE OF THE ASSAY

5.1 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 disposable tips for each specimen.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- The present hPL kit is adjusted to give an absorption for calibrator 4 of 1,200 to 2,000 within 10 minutes at room temperature (22°C). If that absorption value is above the upper performance limit of your microtiterplate spectrophotometer or lower than 1,200, you can reduce or extend the incubation time of the final enzymatic formation of color accordingly. As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions.

5.2 Sample preparation

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

- Add for each sample, which is to be measured, 1ml of Zero Calibrator in one test tube.
- Add 10µl of each sample to the appropriate test tube. Mix all tubes for 10 seconds on an Vortex mixer (avoid foaming).

Procedural 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.
- All calibrators, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

5.3 Assay Procedure

- Secure the desired number of coated *Microtiterwells* in the holder.
- Dispense **100 µl** Anti-hPL *Enzyme-Conjugate* into each well.
- Dispense **10 µl** hPL *Calibrators*, controls and prediluted serum specimen **with new disposable tips** into appropriate wells.
- Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
- Incubate for **30 minutes** at room temperature.
- Briskly shake out the contents of the wells.
- Rinse the wells 5 times with distilled water.
- Strike the wells sharply on absorbent paper to remove residual water droplets.
- Add **100 µl** of *Substrate Solution* to each well.
- Incubate for **10 minutes** at room temperature.

11. Stop the enzymatic reaction by adding **50 µl** of *Stop Solution* to each well.
12. Read the OD at **450±10 nm** with a microtiterplate reader.

Final Reaction Stability

It is recommended that the wells be read within 10 minutes following step 11.

5.4 Calculation of Results

1. Calculate the average absorbance values for each set of reference calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in mg/L 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 of hPL in mg/L from the calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

6 PERFORMANCE CHARACTERISTICS

6.1 Specificity

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

6.2 Sensitivity

The minimal detectable concentration of hPL by this assay is estimated to be 0.3 mg/l.

6.3 Precision

6.3.1 Intra Assay Precision

Within-run precision was determined by replicate determinations of three different control sera in one assay. The within-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	18	18	18
Mean HPL (mg/l)	0.66	2.34	6.24
Standard Deviation	0.04	0.13	0.42
Coefficient of Variation (%)	6.06	5.55	6.73

6.3.2 Inter Assay Precision

Between-run precision was determined by replicate measurements of three different control sera in several different assays. The between-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	39	24	24
Mean HPL (mg/L)	0.68	2.52	6.87
Standard Deviation	0.06	0.18	0.39
Coefficient of Variation (%)	8.82	7.14	5.67

6.4 Recovery and linearity

6.4.1 Recovery

Various patient serum samples of know hPL levels were mixed and assayed in duplicate. The average recovery was 101.17%.

Expected Concentration	Observed Concentration	
mg/l	mg/l	% Recovery
1.51	1.43	94.7
2.60	2.81	108.0
3.38	3.17	93.8
3.97	3.97	100.0
5.84	6.13	105.0
6.76	7.13	105.5

6.4.2 Linearity

Two patient samples were serially diluted with Zero Calibrator in a linearity study.

The average recovery was 99.7%.

Patient		Expected Concentration	Observed Concentration	
Number	Dilution	mg/l	mg/l	% Recovery
1	1:50		14.16	
	1:100	7.08	6.69	94.5
	1:200	3.54	3.52	99.4
	1:400	1.77	1.78	100.6
	1:800	0.88	0.99	112.5
2	1:50		15.97	
	1:100	7.98	6.42	80.5
	1:200	3.99	4.04	101.3
	1:400	1.99	1.99	100.0
	1:800	0.99	1.08	109.1

6.5 Hook effect

In this assay, no hook effect is observed up to 700 mg/l of hPL.

6.6 Quality control

Good laboratory practice requires that controls are 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. Controls containing azide should not be used.

7 LIMITATIONS OF PROCEDURE

1. Reliable and reproducible results will be obtained when the assays procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.