



Human Placental Lactogen (HPL) MICRO-ELISA Test Kit

Product No: T115
Pkg. Size: 96 tests

Description

The MICRO-ELISA PLACENTAL LACTOGEN (hPL) is a solid phase sandwich-type enzyme immunoassay (ELISA) for the quantitative determination of human placental lactogen (hPL) concentration in human serum.

Summary and Explanation of Test

Human placental lactogen (hPL or chorionic somatomammotropin) is a polypeptide produced during pregnancy by placental trophoblastic cells.¹ The level of placental lactogen in maternal serum is directly related to placental function.¹ HPL is detected in the serum of pregnant women about six weeks after conception and its concentration increases gradually to peak levels of approximately 4 to 20 µg/ml by the end of the 34th week.⁷⁻⁹ The hPL concentration in a normal pregnancy increases gradually (without decreases) until the 34th week where it remains stable for the remainder of the pregnancy.^{1,7-10} Consistently low levels throughout pregnancy or a sudden sharp drop in serial determinations are an indication of fetal distress.¹⁰ After normal delivery the hPL concentration falls to an undetectable level rapidly.

HPL is a polypeptide (molecular weight approximately 21,000) which contains certain amino acid sequences similar to growth hormone and prolactin. Its structure is however distinct enough to produce the biological and immunological specificity of the protein. The MICRO-ELISA PLACENTAL LACTOGEN (hPL) utilizes two monoclonal antibodies to distinct determinates of hPL. The use of these antibodies allows the measurement of the concentration of placental lactogen and provides a system with high affinity to hPL and with virtually no cross reactivity with growth hormone or prolactin.

Principle of the Procedure

The MICRO-ELISA PLACENTAL LACTOGEN test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). Antibody (mouse monoclonal) to hPL is coated to a plastic well (solid phase). Antibody (mouse monoclonal) to hPL is contained in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test serum sample is allowed to react simultaneously with the coated and conjugated antibodies, resulting in the hPL molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute incubation at room temperature, the sample well is washed to remove unbound enzyme labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H₂O₂, and tetramethylbenzidine, TMB) is added to the well and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The intensity of the blue color is directly proportional to the concentration of hPL in

the sample.

Reagents

Components in Each 96-Test

Micro-ELISA Placental Lactogen (HPL) Test Kit

1. 96 wells, hPL **ANTIBODY COATED WELLS**: Coated with anti-hPL (mouse monoclonal); 8 strips of 12 wells each (strips may be broken apart as required), contained in a pack with silica gel desiccant.
2. 1 bottle, 22 ml, hPL **ENZYME ANTIBODY CONJUGATE**: anti-hPL (mouse monoclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C Yellow #5 as coloring agent.
3. 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN**: Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
4. 1 bottle, 12 ml, **STOP SOLUTION**: 1 N H₂SO₄.
5. 1 vial, 2 ml, **0 µg/ml hPL CALIBRATOR**: Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
6. 5 vials, 0.75 ml, hPL **CALIBRATORS**: hPL in bovine serum; contains 0.02% thimerosal as a preservative. **0.25 µg/ml, 1.0 µg/ml, 2.5 µg/ml, 5.0 µg/ml and 15 µg/ml.**
7. 1 bottle, 50 ml, **SAMPLE DILUENT**: Buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C Red #40 as coloring agent.
8. 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X)**: Buffered detergent solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

Additional Materials Required

Disposable tip precision pipets - 0.01 ml, 0.2 ml and 0.5 ml.
microtiter well holder.
Microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store all components at 2°-8°C when not in use. Expiration date printed on the kit indicates limits of stability.

The hPL ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2° - 30°C.



Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent instability or deterioration.

Instruments

Performance of the PLACENTAL LACTOGEN (hPL) test requires use of a precision microtiter plate reader with a wavelength of 450 + 20 nm:

Specimen Collection and Preparation

Serum is used in the MICRO-ELISA PLACENTAL LACTOGEN (hPL) test.

Collect blood by venipuncture into plain tubes and separate serum from cells by centrifugation. Transfer the cell-free serum to an appropriate storage tube. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for 24 hours. If the serum is not assayed within 24 hours, store frozen (-20°C) in a tightly sealed tube for up to 2 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Repeated freezing and thawing should be avoided.

Micro-ELISA HPL Procedure

Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

WARNINGS AND PRECAUTIONS FOR THE USER

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. **HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS.** Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Avoid contact with SUBSTRATE-CHROMOGEN (tetramethylbenzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

Do not interchange reagents between test kits with different lot numbers.

Specimen and reagents should be allowed to come to room temperature and mixed thoroughly by gentle inversion or swirling before assay is run.

Azide inhibits this enzyme reaction. Avoid the use of samples or commercial controls that contain azide.

Assay Procedure

1. In a labeled, clean dry test tube; MAKE A 1:51 DILUTION OF THE CALIBRATORS, CONTROLS AND PATIENT SAMPLES BY ADDING 10 µl OF SAMPLE TO 0.5 ml OF DILUENT. Mix by vortexing.
2. Place sufficient MICROTITER WELLS in a holder to run CALIBRATORS, controls and patient samples in duplicate. Record the position of samples on a work sheet.
3. Pipet 10 µl of the DILUTED CALIBRATORS and DILUTED Samples into the corresponding WELL.
4. Pipet or dispense 200 µl of the CONJUGATE into all the wells and mix gently. Note: A mechanical pipettor/dilutor system can be used to simultaneously deliver the diluted sample and conjugate to the test wells.
5. Incubate at room temperature (18 - 30°C) for 60 minutes.
6. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
7. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

8. Repeat step 7 three more times (for a total of 4 washes).
9. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
10. Pipet or dispense 100 µl (0.1 ml) of SUBSTRATE-CHROMOGEN solution into wells.
11. Mix thoroughly and incubate 15 minutes at room temperature (18-30°C).
12. Pipet or dispense 100 µl (0.1 ml) of 1 N H₂SO₄ into each well and mix thoroughly.
13. Read the absorbance of each well at 450 ± 20 nm against water.



Calculation of Results

1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each DILUTED hPL CALIBRATOR on the vertical (Y) axis versus the corresponding hPL concentration (LABEL VALUE) on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding hPL concentration in µg/ml from the curve. The value obtained is already corrected for the 1:51 dilution. Multiply the value by any additional dilution factor if required, i.e., for a 1:2 dilution of the standard 1:51 dilution multiply the answer from the curve by 2.

EXAMPLE DATA

CALIBRATOR	A ₄₅₀	Mean A ₄₅₀	hPL µg/ml
0.0 µg/ml	0.024, 0.028	0.026	
0.25 µg/ml	0.054, 0.051	0.052	
1.0 µg/ml	0.149, 0.141	0.145	
2.5 µg/ml	0.398, 0.386	0.392	
5.0 µg/ml	0.846, 0.858	0.852	
15.0 µg/ml	2.811, 2.747	1.283	

SAMPLES

# 1 (undiluted)	0.052, 0.056	0.054	0.9 **
# 2 (diluted 1:51)	0.899, 0.900	0.900	7.06

**** HIGH DOSE HOOK EFFECT, DO NOT USE UNDILUTED SAMPLES.** Diluted samples with values of >50 µg/ml will not result in a high dose hook effect.

The range of this assay is 0-15 µg/ml. For specimen with hPL concentrations beyond the standard curve (15 µg/ml), repeat the test by diluting the specimen with the DILUENT. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

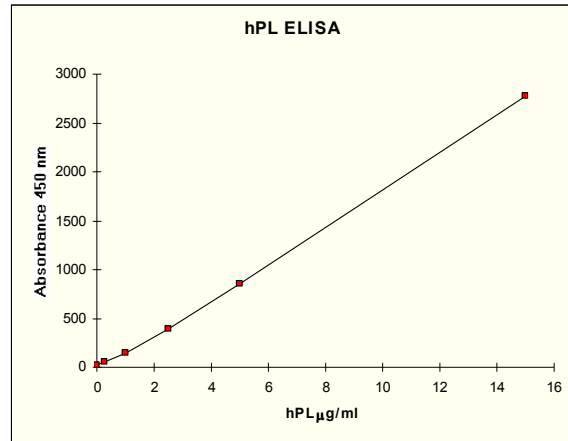
Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls having values at three levels (low, medium and high values) should be used. Controls containing azide can not be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 µg/ml Calibrator exceeds 0.050 it is an indication of careless washing and the assay must be repeated.

EXAMPLE CALIBRATION CURVE.

DO NOT USE IN PLACE OF CURVE DETERMINED AT TIME OF ASSAY.



Standardization

The PLACENTAL LACTOGEN (hPL) CALIBRATORS have been standardized against the World Health Organization First International Reference Preparation (1st IRP #73/545). 1 µIU/ml is equal to 1 µg/ml.

Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. All reagents should be allowed to come to room temperature prior to beginning the assay.
3. When it is necessary to measure levels of hPL greater than the 15.0 µg/ml CALIBRATOR, the sample should be diluted with DILUENT and reassayed. **** HIGH DOSE HOOK EFFECT. DO NOT USE UNDILUTED SAMPLES.** Diluted samples with values of >50 µg/ml will not result in a high dose hook effect.
4. The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.
5. FINAL REACTION STABILITY: Because the color development reaction is not stopped, the spectrophotometric measurement should be made within thirty minutes after the addition of the SUBSTRATE-CHROMOGEN solution.
6. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
7. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies.¹⁵⁻¹⁷ Serum samples from patients who have received



infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the MICRO-ELISA hPL test as erroneous results may be obtained.¹²⁻¹⁴ These conditions should be ruled out prior to clinical evaluation of test results.

Expected Values

hPL is not normally detected in the serum of healthy men and healthy non-pregnant women. The concentration of hPL in the serum of pregnant women is detectable six week post implantation and continues increasing to 4 - 20 µg/ml during the first 34 weeks, reaching a maximum around the 34th week and remaining constant until delivery. The following levels have been reported in the literature.^{1,5,7,11}

Gestational age (Weeks)	Mean hPL (µg/ml)	95% confidence interval
24	3.0	2.0 - 4.0
26	3.1	2.5 - 4.9
28	4.6	2.9 - 5.9
30	4.7	3.1 - 6.9
32	5.4	3.5 - 7.8
34	6.2	3.7 - 8.8
36	6.3	3.8 - 9.2
38	6.8	3.9 - 9.5
40	6.5	4.0 - 9.7
42	6.4	4.0 - 9.8

It is recommended that each laboratory determine its own normal range.

Comparison to Other Quantitative Serum hPL Assays

Correlation studies on a random group of 60 serum samples with a range of values from 0 - 14.8 µg/ml, were performed using the quantitative results from the PLACENTAL LACTOGEN (hPL) Test and another hPL RIA Test (DPC). The following results were obtained:

	Slope	Y-Intercept	Correlation Coefficient
n= 60	1.011	0.018	0.987

Specificity

Studying the interference of chorionic gonadotrophin (hCG), LH, FSH, TSH, prolactin and growth hormone in serum samples determined specificity of this test system. The cross reactivity with hCG, LH, FSH and TSH was 0%, with prolactin and growth hormone less than 0.0001%.

Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean ± two standard deviations for twenty-one replicates at the zero point of standard curve. The calculated sensitivity is 0.1 µg/ml.

Within-Run Precision

The intra-assay precision was determined by measurement of three sets of pooled sera in assay replicates of 21 in a single run.

SERUM	A	B	C
n=21			
Mean (µg/ml)	0.66	3.30	7.40
S.D.	0.034	0.15	0.27
C.V.%	5.15	4.55	3.65

Between-Run Precision

The inter-assay precision was determined by duplicate measurements for three serum pools in twenty separate runs.

SERUM	A	B	C
n=20			
Mean (µg/ml)	0.65	3.32	7.29
S.D.	0.06	0.17	0.43
C.V.%	9.23	5.12	5.90

Dilution Study

The linearity of the MICRO-ELISA PLACENTAL LACTOGEN (hPL) test system was determined by diluting patient serum samples with high hPL values with a male sample with an hPL value of 0.0 µg/ml hPL.

Dilution Factor	Expected Value	Observed Value	% of Expected Value
1:51	-	12.6	-
1:2	6.30	6.45	102.4
1:4	3.15	3.28	104.1
1:8	1.58	1.75	110.7
1:16	0.79	0.69	87.3
1:32	0.39	0.36	92.3
1:64	0.20	0.22	110.0

Recovery Studies

Three normal patient sera with known hPL values were spiked with 1.0, 6.0 and 12.0 µg/ml of hPL. The samples were assayed in duplicate.

	hPL added (µg/ml)	Expected Value	Recovered Value	% Recovery
Patient 1 (2.99 µg/ml)	1.0	3.99	3.82	95.7
	6.0	8.99	8.45	93.9
	12.0	14.99	14.23	94.9
Patient 2 (4.76 µg/ml)	1.0	5.76	6.04	99.1
	6.0	10.76	11.21	104.2
	12.0	16.76	>15.	-
Patient 3 (0.00 µg/ml)	1.0	1.00	0.87	87.0
	6.0	6.00	6.52	108.6
	12.0	12.00	12.47	103.9

The average recovery was 98.4%.



References

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