

PROLACTIN ENZYME IMMUNOASSAY TEST KIT

Catalog Number: 6107305



Enzyme Immunoassay for the Quantitative Determination of Prolactin Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Prolactin Enzyme Immunoassay

INTENDED USE

For the quantitative determination of prolactin concentration in human serum.

INTRODUCTION

Prolactin (PRL) is a polypeptide hormone secreted by the anterior pituitary gland. Release of prolactin is pulsatory, and regulated by hypothalamic releasing and inhibitory factors. High prolactin levels are observed during pregnancy and the post-partum-lactation period. Other physiological states associated with high prolactin levels include physical and emotional stress, sleep and hypoglycaemia. Hyperprolactinaemia can cause suppression of gonadal function. Prolactin measurement therefore forms an essential part of investigations of infertility. In women, symptoms that may accompany hyperprolactinaemia include nonpuerperal galactorrhoea, amenorrhoea and other menstrual disorders. In men it may be associated with loss of libido and impotence. Pathological causes of hyperprolactinaemia include prolactin-secreting macro- and microadenomas, hypothyroidism and renal failure.

PRINCIPLE OF THE TEST

The ELISA test is performed as an indirect solid phase sandwich-type immunoassay. Microwells are coated with anti-monoclonal prolactin followed by blocking the unreacted sites to reduce non-specific binding.

- Step 1 Prolactin Antigens present in calibrators and patient samples bind to the coated antibody.
- Step 2 The Antigen-Antibody complex is reacted with enzyme (HRP) labeled anti-monoclonal prolactin conjugate resulting in the monoclonal prolactin antigen being sandwiched between the solid phase antibody and the enzyme conjugate.
- Step 3 The enzyme converts added substrate (TMB) to form a colored solution.
- Step 4 The intensity of color change, which is proportional to the concentration of Antibodies present in the samples is read by a microplate-reader at 450 nm. Results are expressed in nanogram per milliliter (ng/mL).

REAGENTS

Materials provided with the kit:

- **Microwell plate.** 12x8 well strips. Individually separable wells. Coated with monoclonal Anti-Prolactin., packaged in an aluminum bag with a drying agent.
- **Calibrators.** 5 Vials x 0.2 mL.
Concentration of Progesterone :

ng/mL	0	5	20	75	200
- **Enzyme-antigen Conjugate.** Prolactin-horseradish peroxidase (HRP) conjugate. 12 mL.
- **TMB Substrate solution.** H₂O₂-TMB 0.25 g/L (avoid any skin contact). 12 mL
- **Stop Solution.** Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL.

PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision $\geq 1.5\%$) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

STORAGE OF TEST KITS

The components will remain stable through the expiration date shown on the label if stored between 2-8°C in dark. Do not freeze. Do not use reagents beyond the kit expiration date.

The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells.

Once opened the bag, microplate strips are stable for 1 month at 2-8°C in the plastic bag tightly sealed, with the silicagel.

Opened reagents are stable for 1 month at 2-8°C.



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REAGENT PREPARATION

- Stable until the expiration date of the kit when stored at 2-8°C.
- Coated microwell strips are for one time use only.
- Calibrators, Enzyme Conjugate, Substrate Solution and Stop Solution are ready to use and need not to be diluted.

SPECIMEN COLLECTION AND PREPARATION

The determination of Prolactin can be performed in plasma as well as in serum.

Store specimens at 2°- 8°C for up to a maximum of 2 days. For longer storage, specimens should be frozen. Avoid repeated freezing and thawing of samples.

Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Neither Bilirubin nor Hemolysis have significant effect on the procedure.

Usually no dilution necessary; for samples with concentration more than 200 ng/ml, dilute the sample 1:1 with Standard.

PRECAUTIONS

- Do not use beyond expiration date on the label.
- Do not use if reagent is not clear or if a precipitate is present.
- Do not interchange kit components.
- Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling.

ASSAY PROCEDURE

- Allow all the reagents and samples to reach room temperature (20-25°C) before running the assay.

Format the microplates' wells for each serum reference, control and patient specimen to be assayed according to the following figure. 5 calibrators (standards) (SA-SE) and 1 Blank should be included. The user has the option to run Patient Samples (P) in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
a	B	SD	P3									
b	SA	SE	P4									
c	SA	SE	P4									
d	SB	P1	P..									
e	SB	P1	P..									
f	SC	P2										
g	SC	P2										
h	SD	P3										

Cal.	Conc. ng/mL
SA	0
SB	5
SC	20
SD	75
SE	200

- Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder.
- Pipette 10 µL of Calibrators and 10 µL Patient Samples into the wells and incubate 15 minutes at room temperature.
- Add 100 µL Enzyme Conjugate to the wells except for Blank well and incubate 30 minutes at room temperature.
- Add app. 300µL of distilled water, decant (tap or blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes.
- Pipette 100 µL of Substrate Solution into each microwell in the same order and timing as for the Enzyme Conjugate, Blank well included.
- Incubate 10 minutes at room temperature. Hereby take care to shake the plate casually. This insures the lowest possible CV values.
- Add 100 µL of Stop Solution into each microwell using the same order and timing as for the addition of the Substrate Solution.
- Read absorbance of each microwell at 450 nm against blank using a microplate reader. The developed color is stable for at least 30 minutes. Read optical densities during this time.

CALCULATION OF RESULTS

Mean absorbance and relative percentage

- Calculate the mean of the absorbances (Em) corresponding to the single points to the standard curve and of each sample.
- Subtract the mean absorbance value of the zero standard from the mean absorbance values of standards and samples.
- Draw the standard curve on log-log or lin-lin graph paper by plotting absorbance values of standard against appropriate Prolactin concentration.
- Read off the Prolactin concentrations of the control and samples.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.

The serum or plasma Prolactin values are comprised in the following intervals:

Sample	Range ng/mL
Male	4.0 – 12.0
Female: menstrual cycle1	2 - 22.0
Menopause	0.7 - 17

Some of the female population tested in this group were probably using oral contraceptives, which may affect results.



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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. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. 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.

PERFORMANCE CHARACTERISTICS

A. Sensitivity

The minimal detectable concentration of Human Prolactin by this assay is estimated to be 1.0 ng/mL.

B. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

hProlactin	100.0 %
hGH	< 0.2 %
HPL	< 0.1 %

C. Precision

Intra Assay variation

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

Sample	1	2	3
Number of replicates	16	16	16
Mean Prolactin (ng/mL)	7.5	28.2	64.2
Std. Deviation	0.45	1.8	4.25
Coef. of Variation (%)	6.1	6.4	6.9

Inter Assay variation

Between-run precision was determined by replicate determination of three different controls in one assay. The between assay variability is shown below:

Sample	1	2	3
Number of replicates	16	16	16
Mean Prolactin (ng/mL)	7.7	27.4	59.3
Std. Variation	0.51	2.07	5.15
Coef. of Variation (%)	6.6	7.6	8.7

D. Recovery

Various patient samples of known Prolactin levels were combined and assayed in duplicate. The average recovery 98.2% with reference to the original concentrations.

Expected conc.	Observed conc.	Recovery
9.4	8.9	94.7
15.2	14.7	96.7
26.6	24.7	92.8
57.5	60.3	104.8
98.3	104.9	106.7

E. Linearity

Two patient samples were serially diluted with zero standard in a linearity study. The average recovery was 102.1 %.

Patient	Exp. Conc	Obs. Conc	Recovery
1		54.4	
Dil. 1 / 2	27.2	28.6	105.1
Dil. 1 / 4	13.6	13.0	95.6
Dil. 1 / 8	6.8	7.2	105.9
2		38.8	
Dil. 1 / 2	19.4	18.5	95.3
Dil. 1 / 4	9.7	9.9	102.1
Dil. 1 / 8	4.9	5.3	108.1

F. Limitations of the procedure

In this assay, no hook effect is observed up to 4000 ng/ml of Prolactin.

REFERENCES

1. Wisdom, G.B. Clin. Chem. 22/8 1243 - 1255 (1976)
2. Shome, B. and Parlow, A.F., J. Clin. Endocr. Metab.,39, 199 – 202 (1974).
3. Shome, B. and Parlow, A.F., J. Clin. Endocr. Metab.,39, 203 – 205 (1974).
4. Uotila, M. Ruoslahti, E. Engvall, E., J. Immunol. Methds, 42, 11-15 (1981)
5. Acosta, A.A.M.D. and Wright, G.L., Journal of Clinical Immunoassays, 6, 41 (1983).

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