



Prolactin (human) ELISA Kit

(Catalog # K4687-100, 100 assays; Store at 2-8°C)

5/14

I. Introduction:

Prolactin (PRL) is secreted from the anterior pituitary gland in both men and woman. Women normally have slightly higher basal prolactin levels than men. During and following pregnancy, prolactin, in association with other hormones, stimulates breast development and milk production. Hypersecretion of prolactin can be caused by pituitary tumors, hypothalamic diseases, hypothyroidism, renal failure, acute exercise and several medications. Hyperprolactinemia inhibits hypogonadism in men and women with accompanying low FSH and LH levels. BioVision's Prolactin kit is a solid phase direct sandwich ELISA Kit. The samples, and anti-Prolactin HRP conjugate are added to the wells coated with monoclonal antibody to Prolactin. Prolactin in the sample binds to anti-Prolactin Mab on the well and the anti-Prolactin HRP then binds to Prolactin. Unbound Protein and HRP conjugate is washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of Prolactin in the samples. A standard curve is prepared relating color intensity to the concentration of the Prolactin.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human Prolactin

IV. Sample Type:

- Serum

V. Kit Contents:

Components	K4687-100	Part No.
Microplate coated with Prolactin MAb, 96 wells	12 stripsx8 wells	K4687-100-1
Prolactin Standard: (0.5 ml) (ready to use)	6 vials	K4687-100-2
Enzyme Conjugate (ready to use)	12 ml	K4687-100-3
Wash Concentrate (20X)	25 ml	K4687-100-4
TMB Substrate (ready to use)	12 ml	K4687-100-5
Stop Solution (ready to use)	12 ml	K4687-100-6

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

- **Wash Concentrate:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

VIII. Warning & Precautions:

- Potential biohazardous materials: The calibrator & controls contains human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- This test kit is USA FDA exempt product.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 5 days. Store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Don't use grossly lipemic specimens.

X. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use. Check Prolactin standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 25 µl of Prolactin Standard, control, and samples into designated wells.
3. Add 100 µl of enzyme conjugate to all wells. Shake gently for 20-30 sec. to mix.
4. Cover the plate and incubate for 60 min. at room temperature (18-26°C).
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells & incubate for 15 min. at room temperature.
7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stop solution.



- XI. Calculation:** Construct the standard curve; plot the absorbance for the Prolactin standards (vertical axis) versus Prolactin standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve:

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	0.037
Standard 2 (5 ng/ml)	0.363
Standard 3 (10 ng/ml)	0.648
Standard 4 (25 ng/ml)	1.181
Standard 5 (50 ng/ml)	1.647
Standard 6 (100 ng/ml)	2.353

Expected Values: It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for Prolactin were established by the BioVision and may be used as initial guideline ranges only

Classification	Normal Range (ng/ml)
Males	2-17
Females	3-25
Pregnancy 3 rd trimester	95-480

Performance Characteristics:

- 1. Correlation with a reference ELISA Kit:** A total of 110 sera were tested by BioVision Prolactin ELISA & a reference ELISA kit. Results are as follows:

Correlation	Slope	Intercept
0.86	1.96	4.81

2. Precision:
Intra-Assay:

Sample	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	33.2	2.27	6.8
2	16	15.7	0.75	4.8
3	16	4.2	0.24	5.8

Inter-Assay:

Sample	No. of runs	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	10	30.5	2.7	6.9
2	10	14.5	0.98	6.7
3	10	4.3	0.3	6.9

- 3. Sensitivity:** The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Sample	No. of Replicate	Mean ng/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.126	0.208	0.334 ng/ml

- 4. Recovery:** Known quantities of prolactin were added to serum that contained low concentration of Prolactin.

Expected Value (ng/ml)	Recovered (ng/ml)	% of Recovery
5	4.8	96
15	15.5	103.3
30	32	106.7

- 5. Linearity:** Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. Prolactin values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (ng/ml)	% of Recovery		
		1:2	1:4	1:8
1	60	102	98	92
2	50	105	97	93

XII. RELATED PRODUCTS:

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| Prolactin (mouse/rat) ELISA Kit (4688) | Progesterone (human) ELISA Kit For Saliva (7416) |
| Testosterone (human) ELISA Kit (K7417) | Progesterone (human) ELISA Kit (7414) |
| Prolactin, human recombinant (4687) | Progesterone (mouse/rat) ELISA Kit (7415) |
| Prolactin, rat recombinant (4689) | Testosterone (mouse/rat) ELISA Kit (K7418) |
| Prolactin Antibody (5688, 5689, 6649) | Prolactin, murine recombinant (4688) |

FOR RESEARCH USE ONLY! Not to be used on humans.