



Prolactin (mouse/rat) ELISA Kit

(Catalog # K4688-100, 100 assays; Store at 2-8°C)

5/14

I. Introduction:

Prolactin is secreted from the anterior pituitary gland. Plasma and pituitary PRL levels are significantly greater in adult female than in male rats. This difference is thought to be brought about by ovarian steroids. A similar sex difference was found in pituitary PRL content in normal mice from 30 days of age. 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. BioVision's Prolactin kit is a solid phase sandwich ELISA Kit. The samples and biotinylated anti-prolactin antibodies are added to the wells coated with polyclonal antibody to prolactin. Prolactin in samples binds to the anti-prolactin polyclonal antibody on the well and the biotinylated anti-prolactin antibody binds to prolactin. Unbound protein and biotin conjugate are washed off by wash buffer. Horseradish peroxidase conjugated streptavidin is added to each wells and the streptavidin is allowed to bind to the biotin in the complex. Unbound enzyme conjugate is washed off. 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:

Mose/Rat Prolactin

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K4688-100	Part No.
Microplate coated with Prolactin MAb, 96 wells	12 stripsx8 wells	K4688-100-1
Prolactin Standard: (0.5 ml) (ready to use)	6 vials	K4688-100-2
Biotinylated Antibody reagent (ready to use)	12 ml	K4688-100-3
Streptavidin Enzyme Conjugate (ready to use)	12 ml	K4688-100-4
Wash Concentrate (20X)	25 ml	K4688-100-5
TMB Substrate (ready to use)	12 ml	K4688-100-6
Stop Solution (ready to use)	12 ml	K4688-100-7

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:

- 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 plasma 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 sample should be completely thawed and mixed well.

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 50 µl of Prolactin Standards, control, and samples into designated wells.
3. Add 100 µl of biotin conjugate to all wells. Shake gently for 10 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 streptavidin enzyme conjugate to all wells. Cover the plate & incubate for 30 min. at room temperature.
7. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
8. Add 100 µl of TMB substrate to all wells & incubate for 15 min. at room temperature.
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. 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)	0.07
Standard 2 (3 ng/ml)	0.19
Standard 3 (6 ng/ml)	0.36
Standard 4 (25 ng/ml)	0.79
Standard 5 (100 ng/ml)	1.45
Standard 6 (200 ng/ml)	2.01

Performance Characteristics:

1. Precision:
Intra-Assay:

Sample	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	30.1	2.1	6.9
2	16	16.2	0.8	4.9
3	16	5.1	0.3	5.9

Inter-Assay:

Sample	No. of runs	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	10	32.2	2.1	6.5
2	10	16.7	0.88	5.3
3	10	5.2	0.4	7.7

- 2. 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	0.10	0.05	0.2 ng/ml

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

Expected Value (ng/ml)	Recovered (ng/ml)	% of Recovery
5	4.6	92
20	19.6	98
40	42	105

- 4. 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:

Sample	Original Value (ng/ml)	% of Recovery		
		1:2	1:4	1:8
1	60	99	96	93
2	50	97	95	94

XII. RELATED PRODUCTS:

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| Prolactin (human) ELISA Kit (4687) | 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.