Prepared in August 2008
PRB021

Pana-test Rat Prolactin

1. Introduction

Prolactin, one of the a protein hormones, having a molecular weight of 20 kDa, is known to secreted from the anterior lobe of the pituitary gland, enhance the growth of mammary glands and promotes milk secretion in mammals. In animal experiments, the prolactin level in blood can reflect hypophysis adenoma and abnormalities in the secretory modulation mechanism. This kit employs prolactin determination based on the ELISA method. It is possible to determine blood prolactin levels in a short time with a specific antibody to rat prolactin.

2. Characteristics

- This kit includes an exclusive reagent for quantitative determination of prolactin.
- No specific facility is necessary.
- The pro-zone phenomenon could not be noted up to 2000 ng/mL of the level of prolactin.

3. Components of the Kit

- ELISA plate (anti-rat prolactin antibody-coated microplate) ..... 1 plate
- Standard rat prolactin (50 ng/mL) for 1 mL (lyophilized) ..... 1 vial
- Sample diluent, 40 mL ..... 1 vial
- Enzyme-labeled antibody (peroxidase-conjugated anti-rat prolactin antibody) for 12 mL (lyophilized) ..... 1 vial
- Chromogen solution (containing 13.2 mg of 3,3',5,5'-tetramethylbenzidine in 0.5 mL of *N,N*-dimethylformamide) ..... 1 vial
- Substrate solution, 20 mL (containing 0.0083 w/v% hydrogen peroxide) ..... 1 vial
- Washing buffer concentrate, 40 mL (10-fold concentrated PBS-Tween 20, for 400 mL use) ..... 1 vial
- Stop solution, 15 mL (1 mol/L sulfuric acid) ..... 1 vial

4. Reagent Preparation

Component	Preparation	Reagent prepared	Storage condition and stability
① ELISA plate	Wait until the plate reaches to room temperature. Add 300 µL of wash buffer to each well just before use, and leave for 10 minutes.	Anti-rat prolactin antibody-coated plate	Prepare a required number of strip only immediately before use.
② Standard rat prolactin	Add accurately 1.0 mL of purified water [1] to the vial, and mix it thoroughly for complete dissolution. Be careful not to from bubbles.	Standard rat prolactin (50 ng/mL)	Stable in a refrigerator (2 to 10°C) for one week
③ Sample diluent	Use it as it is		Stable in a refrigerator (2 to 10°C) for one week
④ Enzyme-labeled antibody	Add accurately 12 mL of purified water to the vial, and mix it thoroughly.	Enzyme-labeled antibody solution	Stable in refrigerator (2 to 10°C) for one week
⑤ Chromogen solution ⑥ Substrate solution	Add 100 µL of the chromogen solution into 10 mL of the substrate solution.	Chromogenic substrate solution	Freshly prepare, just before use.
⑦ Wash buffer concentrate	Add the entire volume of the concentrate (40 mL) into 360 mL of purified water, and mix it thoroughly.	Wash buffer [2]	Stable at room temperature for one week
⑧ Stop solution	Use it as it is		Stable at room temperature

1) Distilled or deionized water
2) PBS containing 0.05 v/v% Tween 20

NOTE: *: Distilled or deionized water
All reagents should be allowed to equilibrate to room temperature before use.
Disused strips should be closed up in the foil pouch and stored at 2 to 10°C under dark.
Immediately use the chromogenic substrate solution after mixing ⑤ with ⑥.

5. Supplies Required

- Micropipettes and pipette tips (20 µL, 100 to 1,000 µL)
- Blowout pipettes (1 mL, 10 mL)
- Graduated cylinder (500 mL)
- Squirt bottle, manifold dispenser, or automated microplate washer
- Multi-channel pipette
- Microplate reader capable of measurement at or near 450 nm
- Distilled or deionized water

6. Assay Procedure

6.1 Preparation of Standard Rat Prolactin Solutions

Reconstitute the standard rat prolactin with accurately 1.0 mL of deionized or distilled water, producing 50 ng/mL standard. Swirl or mix gently and leave for a while to ensure complete reconstitution. Make serial dilutions of the 50 ng/ mL standard with the sample diluent, to prepare the standard solutions at 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
Distribute the sample diluent to the 0 ng/mL standard.

6.2 Sample Dilution

Blood sample
Commonly use serum or plasma (containing heparin or EDTA as an anticoagulant) as a sample for measurement of prolactin. Store the samples below -20°C.
If you are suspecting that the prolactin concentration in a test sample exceeds the highest point (50 ng/mL) of the standard curve, we suggest that test sample should be diluted with the sample diluent.

6.3 Assay Protocol

Bring all reagents and samples to room temperature before use. It is recommended that all samples, including the standards, are assayed in duplicate.

1) Add 300 μL of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (no adverse effect, even if left standing for up to 30 minutes.)
2) Aspirate each well to remove the solution.
3) Add 100 μL of the enzyme-labeled antibody and 20 μL of the standard rat prolactin solution or unknown samples to each well, and incubate for 2 hours at room temperature.
4) Aspirate each well and wash the wells with wash buffer (300 μL/well). Repeat the washing procedure further twice. Complete removal of an aqueous fluid in each wash is essential to good performance.
5) Add 100 μL of the chromogenic substrate solution to each well and incubate at room temperature for 30 minutes.
6) Add 50 μL of the stop solution to each well.
7) Measure the absorbance at 450 nm (A_{450}) with a microplate reader.

7. Data Calculation

1) Average the duplicate reading for each standard and each sample.
2) Plot the values of A_{450} (Y-axis) versus the concentrations of the standard solutions (X-axis), thus draw a standard curve.
3) Apply an A_{450} value of each sample in the standard curve, so as to read a prolactin concentration in the sample.
4) In case of a diluted sample, multiply the prolactin concentration by the dilution factor to get a prolactin concentration in the original sample (serum, plasma).

8. Safety Warnings and Precautions

- Strictly observe the storage condition for each reagent.
- All reagents should be brought to room temperature before use.
- Use reagents after confirming complete dissolution and uniformity.
- Take care to not inflict damage on any well when aspirating the solution in each well.
- When measuring many samples in one assay batch, the time period of each reaction for all samples should be uniformed at a fixed time as designated.
- Prepare the standard curve freshly for every measurement.
- Prepare the substrate solution with a clean vessel.
- White powder may sometimes be found in the wells of the ELISA plate. This is due to the dried blocking solution, but will have no effect on the measurement.
- Take care to handle the stop solution, very harmful.

9. Performance Characteristics

9.1 Quantitative Range

0.78 – 50 ng/mL of rat prolactin

9.2 Intra - assay Precision

Standards

Rat prolactin (ng/mL)	(Replicate)	A_{450} (mean)	C.V. (%)
0	(N=8)	0.029	17.2
0.78	(N=8)	0.044	9.1
1.25	(N=8)	0.066	4.5
3.13	(N=8)	0.107	4.7
6.25	(N=8)	0.195	6.7
12.5	(N=8)	0.370	4.3
25	(N=8)	0.690	5.5
50	(N=8)	1.212	2.3

Samples

Sample	(Replicate)	A_{450}		Prolactin conc.(ng/mL)	
		mean	CV (%)	mean	CV (%)
A	(N=8)	0.101	5.9	2.00	12.5
B	(N=8)	0.306	3.3	9.71	2.8
C	(N=8)	0.716	3.6	25.77	4.6

C.V. = coefficient of variation

Sample A: 10-fold dilution of rat serum specimens (male, 7 weeks of age)
Sample B and C: Sample A spiked with standard rat prolactin

9.3 Inter-assay Precision

Standards

Rat prolactin (ng/mL)	(Replicate)	A_{450} (mean)	C.V. (%)
0	(N=8)	0.029	17.2
0.78	(N=8)	0.054	14.8
1.25	(N=8)	0.076	14.5
3.13	(N=8)	0.124	9.7
6.25	(N=8)	0.223	9.9
12.5	(N=8)	0.414	8.9
25	(N=8)	0.718	10.9
50	(N=8)	1.244	8.5

Samples

Sample	(Replicate)	A_{450}		Prolactin conc. (ng/mL)	
		mean	CV (%)	mean	CV (%)
A	(N=8)	0.106	12.3	1.72	14.9
B	(N=8)	0.313	11.5	8.82	9.4
C	(N=8)	0.714	13.2	24.14	10.3

C.V. = coefficient of variation

Sample A: 10-fold dilution of rat serum specimens (male, 7 weeks of age)
Sample B and C: Sample A spiked with standard rat prolactin

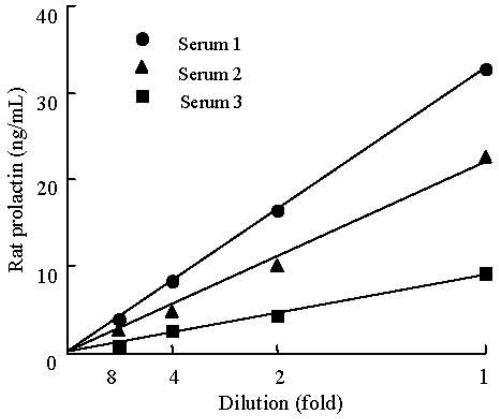
9.4 Recovery

Samples were prepared by spiking the three levels of standard rat prolactin into rat sera (male, 7 weeks of age)

Sample	Spiked amount (ng/mL)	Measured value (ng/mL)	Expected value (ng/mL)	Recovery (%)
Serum 1	0	0.9	-	-
	3.13	4.2	4.0	104.0
	12.5	14.4	13.4	107.3
	25	26.3	25.9	101.7
Serum 2	0	14.1	-	-
	3.13	17.1	17.2	99.1
	12.5	26.9	26.6	101.0
	25	35.1	39.1	89.8

9.5 Linearity of Dilution

Samples were prepared by a serial dilution of a rat serum with the sample diluent up to 8-fold.



10. Storage and Expiry

Store all reagents at 2-10°C under dark and use until a stated expiration date (one year after manufactured).

11. Package

96 tests per kit

Distributed by:

Xceltis

Xceltis GmbH
Im Tal 12
74909 Meckesheim / Germany
Tel.: +49-6226-9724-18
Fax: +49-6226-9724-19
E-mail: info@xceltis.de

＜Manufactured by＞
Mitsubishi Chemical Safety Institute Ltd.
Kumamoto Laboratory
1285 Kurisaki-machi, Uto, Kumamoto 869-0425, Japan