KAMIYA BIOMEDICAL COMPANY

Rat Prolactin ELISA

For the *in vitro* determination of rat Prolactin (rPRL) in serum or plasma

Cat. No. KT-203

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Rat Prolactin ELISA is for the *in vitro* determination of rat prolactin (rPRL) levels in serum or plasma. For research use only.

PRINCIPLE
The ELISA technique uses two antibodies with high affinity but with specificity for two different epitopes on rat prolactin. The first mouse anti-rPRL monoclonal antibody is bound to the polystyrene well and will capture rPRL in the sample in the presence of a second mouse anti-rPRL monoclonal antibody conjugated to horseradish peroxidase (HRP).
Following the incubation and the one step formation of the solid phase-rPRL-conjugated monoclonal antibody sandwich, the well is washed to remove unbound conjugated antibody. Then the TMB substrate is added, which turns from pink to blue proportionally to the rPRL concentration in the sample. Addition of the stop solution turns the color to yellow and the intensity of the yellow color is measured using a spectrophotometer with a 450 nm filter. Sample concentrations are read from a calibration curve and the results are expressed in ng/mL.

COMPONENTS
- Microtiter Plate: 96-well microtiter plate coated with mouse anti-rPRL monoclonal antibody. Allow the microwells/microplate to reach room temperature (RT) before opening the bag. Single use strips/wells.
- Conjugate: 12 mL x 2; horseradish peroxidase (HRP) conjugated mouse anti–rPRL monoclonal antibody, diluted in buffer containing a yellow dye.
- Calibrator Concentrate: 1 vial of Rat prolactin lyophilized in buffer. The Calibrator Concentrate is calibrated against an internal reference preparation of rPRL. The concentration of the Calibrator Concentrate (ng/mL) is printed on the vial label.
- Controls: 2 vials (1 x Control 1, 1 x Control 2) of rPRL lyophilized in buffer. The Controls should be assayed with the samples and the results compared with those printed on the vials.
- Diluent Buffer: 10 mL x 2; Diluent buffer containing preservative (Proclin 0.05%).
- TMB Substrate: 12 mL x 2; TMB Substrate (tetramethylbenzidine) in buffer containing pink dye. **Light sensitive, protect from light.**
- Stop Solution: 10 mL; 2 M H_2SO_4
- Wash Buffer Concentrate: 60 mL; 20X wash buffer solution containing preservative (Proclin 0.05%).

MATERIALS REQUIRED BUT NOT PROVIDED
- Test tubes for dilutions and a tube holder
- Vortex mixer
- Manual or automated precision micropipettes with single use tips for dispensing samples or reagents without cross-contamination
- Multi-channel micropipette or repeating dispenser (Eppendorf type)
- Vacuum pump connected through a trap for aspiration
- 96-well microplate reader with a 450 nm filter
- Semi-logarithmic paper (or software package)
- Microplate washer (facultative)

STORAGE

Store at 4°C. The material can be used up to the expiration date printed on the box label. See preparation section below for storage of prepared solutions. Store unused microtiter strips at 4°C with the desiccant pack in a tightly sealed bag.

PREPARATION

Bring all reagents to RT before use. Before use, reconstitute the contents of the Calibrator Concentrate with 3 mL of Diluent Buffer and the Controls with 0.5 mL of Diluent Buffer. Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing. If not used immediately after reconstitution, store aliquots at -20°C for up to 4 weeks.
Dilute the Wash Buffer Concentrate 1:20 by mixing with 1,140 mL of distilled water and mix well. Diluted Wash Buffer can be stored at 4°C or 25°C.
All reagents are ready for use, except the Wash Buffer Concentrate, Calibrator Concentrate and Controls. After use, close all reagent vials and bottles and store as directed.

Preparation of the Calibrators 0-7

Example of dilution scheme. The exact concentration of the Calibrator is printed on the vial label.

Cal#	Volume of calibrator (µL)	Volume of Diluent Buffer	Conc. ng/mL
7	Calibrator stock tube	3,000 µL	180
6	500 µL of CAL 7	500 µL	90
5	200 µL of CAL 7	600 µL	45
4	100 µL of CAL 7	900 µL	18
3	50 µL of CAL 7	950 µL	9
2	25 µL of CAL 7	975 µL	4.5
1	25 µL of CAL 7	1,975 µL	2.25
0	-	2,000 µL	0

METHOD

Collection and Handling of Serum and Plasma Samples

The blood sample may be collected either into a dry tube or one containing an anti-coagulant. If heparin is used, only the minimum required to avoid clotting should be added.
The serum or plasma, when separated from the red blood cells, may be assayed immediately, within 24 hours if stored at 4°C, or after periods up to several months if stored at -20°C.
Repeated freeze/thaw cycles must be avoided.

ASSAY PROCEDURE

Do not mix reagents from different lots. Bring the different components of the kit to RT prior to use. Perform the assay in duplicates. Calibrators, Controls and samples must be assayed at the same time. Strictly follow the different steps of the procedure and use disposable tips.

Select the number of coated wells needed for the rPRL assays. Store extra wells/strips in the bag along with the desiccant pack and seal tightly.

1. Dispense 10 µL of each diluted Calibrator into appropriate wells.
2. Dispense 10 µL of samples or Control into appropriate wells.
3. Add 200 µL of Conjugate into each well.
4. Incubate for 180 minutes at RT (25°C) without shaking.
5. Flick out the contents of the wells over a basin containing bleach water or aspirate with an automated plate washer.

6. Using the diluted Wash Buffer, wash the wells **six** times with an automated system set to dispense 250 µL per well, or by adding 250 µL to each well, flicking out over a basin and blotting the wells on absorbent paper to remove any residual liquid after each washing.

7. Dispense 200 µL of TMB Substrate solution into each well.
*Note: The TMB Substrate solution should initially be pale at this step. A blue color indicates that it has been contaminated.

8. Incubate for 30 minutes at RT without shaking.

9. Stop the reaction by adding 50 µL of Stop Solution to each well.

10. Place the plate on a flat surface, swirl gently to mix contents or use the option « mixing » if your reader has one.

11. Measure the absorbance at 450 nm on a 96-well microplate reader.

ANALYSIS OF RESULTS

Calculation of Concentration

Draw a calibration curve on semilogarithmic paper by plotting mean absorbance (linear scale) obtained for each Calibrator versus its respective concentration expressed in ng/mL (logarithmic scale). rPRL concentrations in sample may be read directly from the appropriate calibration curve.
If a computer is used to calculate the results, the data can be fitted to the appropriate equation: Non-linear regression, Sigmoïdal dose-response or Sigmoïdal dose-response (Variable slope), Polynomial 4th order or spline cubic.

Example of typical assays

	Contents (ng/mL)	ABS 1st duplicate	ABS 2nd duplicate	Mean ABS	Abs/ Abs Max%	Rat PRL (ng/mL)
Calibrator 0	0	0.085	0.088	0.087	2.76	
Calibrator 1	2.25	0.195	0.197	0.196	6.24	
Calibrator 2	4.5	0.285	0.296	0.291	9.26	
Calibrator 3	9	0.501	0.492	0.497	15.82	
Calibrator 4	18	0.861	0.869	0.865	27.54	
Calibrator 5	45	1.589	1.636	1.613	51.35	
Calibrator 6	90	2.395	2.249	2.322	73.93	
Calibrator 7	180	3.090	3.192	3.141	100.00	
Control 1		0.564	0.613	0.589	18.75	11.23
Control 2		1.739	1.762	1.751	55.75	52.08
Sample 1		0.467	0.468	0.468	14.90	8.28
Sample 2		0.905	0.916	0.911	29.00	20.00
Sample 3		1.953	1.904	1.929	61.41	61.42

Example of typical assay performed at controlled temperature of 25°C. Do not use these values for calculations.

Expected Normal Values

It is recommended that each laboratory establishes its own reference values.

Concentration range for normal male subjects was from 6.6 to 24 ng/mL and for normal female subjects was from 14 to 24 ng/mL in our preliminary studies.

LIMITATIONS

Do not use strongly lipemic, hemolyzed, icteric or turbid specimens.

Special care is needed to prevent contamination of the TMB Substrate by the Conjugate. The TMB Substrate should be pink, a blue coloration indicates that the reagent has been contaminated and must be discarded. Substrate degradation is increased at temperatures above 25°C.

The well washing procedure is critical for the successful performance of the test.

The TMB Substrate is extremely sensitive to certain handling and storage conditions. Avoid exposure to light, heat and contamination with metal ions or peroxidase.

QUALITY CONTROL

Use the controls provided for each assay.
If, in normal assay conditions, the controls are out of the acceptable ranges, the sample results can not be validated. Please contact the manufacturer.

PERFORMANCE CHARACTERISTICS

Specificity

Prolactin from other species can cross-react partially in this assay. Cross-reactivity with other rat pituitary hormones as measured by radioimmunoassay:

Compound	Cross-reactivity (%)
Rat PRL	100.0 %
Rat TSH	<0.1 %
Rat LH	<0.1 %
Rat FSH	<0.1 %
Rat GH	<0.1 %
Mouse PRL	<0.1 %

Analytical sensitivity

The minimum detectable concentration of rat prolactin has been assayed at 0.36 ng/mL and corresponds to the concentration given by two standard deviations above the mean ABS of 25 replicate determinations of the zero Calibrator.

Precision

	Repeatability	Intra-Assay Variation
	Mean value (ng/mL)	% CV (24 replicates)
Sample 1	1.1	12.56
Sample 2	8.63	6.67
Sample 3	29.48	6.64
Sample 4	53.86	5.97

	Reproducibility	Inter-Assay Variation
	Mean value (ng/mL)	% CV (13 replicates)
Sample 1	2.3	17.6
Sample 2	7.4	9.16
Sample 3	17.58	9.24
Sample 4	56.34	11.55

Recovery Test

When rPRL was spiked to rat serum, the recovery of rPRL ranges from 79.6% to 143.8%

Sample 1:

Added Rat PRL (ng/mL)	-	1.14	2.28	4.56	9.13	18.37	36.52	73.04
Assayed Rat PRL (ng/mL)	4.7	6.34	7.8	10.6	14.8	26	42.5	78.2
Rat PRL recovered (ng/mL)	-	1.64	3.1	5.9	10.1	21.3	37.8	73.5
% Recovery	-	143.8	135.9	129.3	110.6	116.0	103.5	100.6

Sample 2:

Added Rat PRL (ng/mL)	-	1.64	3.28	13.12	26.4	52.5	105
Assayed Rat PRL (ng/mL)	0.35	1.74	2.96	10.84	22.9	48.1	93.7
Rat PRL recovered (ng/mL)	-	1.39	2.61	10.49	22.55	47.75	93.35
% Recovery	-	84.8	79.6	79.9	85.4	91.0	88.9

Linearity: Dilution test

The dilution test (dilution with Calibrator 0) indicates that there is immunological identity between the rPRL present in serum and the rPRL used to create the calibration curve.

Sample 1:

Dilution factor	1	2/3	1/2	1/3	1/4	1/6	1/8	1/12
Expected Rat PRL (ng/mL)	-	16.03	12.03	8.02	6.01	4.01	3.01	2.00
Assayed Rat PRL (ng/mL)	24.05	17.22	10.29	7.53	4.82	3.59	2.11	1.61
% Recovery	-	107.4	85.6	93.9	80.2	89.6	70.2	80.3

Sample 2:

Dilution factor	1	2/3	1/2	1/3	1/4	1/6	1/8
Expected Rat PRL (ng/mL)	-	41.13	30.85	20.57	15.43	10.28	7.71
Assayed Rat PRL (ng/mL)	61.7	38.10	30.05	17.50	12.90	8.40	6.10
% Recovery	-	92.6	97.4	85.1	83.6	81.7	79.1

WARNINGS AND PRECAUTIONS

- Please read this manual carefully prior to use. This assay kit is for *in vitro* research use only.
- The kit must be handled by qualified staff.
- Good laboratory and safety practices are advised.
- ***Warning***: This kit contains TMB substrate (3,3',5,5'-tetramethylbenzidine) which has shown a possible mutagenic effect in experimental animals (mice). Avoid contact with skin and eyes. May cause irritation of eyes, skin and gastrointestinal tract.
- ***Warning***: Animal origin materials are used in this kit. Since no known test can guarantee that such material does not contain infectious agents, these products must be considered as potentially infectious and handled with care.

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