Prl (Mouse/Rat) ELISA Kit

Catalog Number KA0923

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The Prl (Mouse/Rat) ELISA Kit is used for the quantitative measurement of prolactin in Mouse or Rat serum or plasma. For research use only. Not for use in diagnostic procedures.

Background

Prolactin (lactogenic hormone) is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. 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, hypothyroidm, renal failure, acute exercise and several medications.

Principle of the Assay

The Prl (Mouse/Rat) ELISA Kit is based on a solid phase sandwich ELISA. The samples and diluted 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. Diluted 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.

General Information

Materials Supplied

List of component

Component	Amount
Microwell coated with Prolactin polyclonal antibody	96 (12x8) wells
Prolactin Standards (Ready to use)	0.5 mL x 6
Biotinylated Antibody reagent (Ready to use)	12 mL
Streptavidin Enzyme Conjugate (Ready to use)	12 mL
TMB Substrate (Ready to use)	12 mL
Stop Solution (Ready to use)	12 mL
20X Wash concentrate	25 mL

Storage Instruction

- ✓ Store the kit at 2-8°C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- ✓ For Research Use Only. Not for use in diagnostic procedures.
- ✓ For laboratory use.
- ✓ Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- ✓ 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 plasma 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.

- Limitations of the Test

✓ The test results obtained using this kit is for research use. It is recommended that each lab establish normal range based on sample population.

✓ Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Assay Protocol

Reagent Preparation

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 (20-25°C).

Sample Preparation

- ✓ Collect blood specimens and separate the plasma immediately.
- ✓ Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 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.

Assay Procedure

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipet 50 µL of Prolactin standards, control and samples.
3. Add 100 µL of biotin conjugate to all wells. Shake the plate for 10 seconds to mix the solution.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
6. Add 100 µL of streptavidin enzyme conjugate to all wells.
7. Cover the plate and incubate for 30 minutes at room temperature.
8. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Add 100 µL of TMB substrate to all wells.
10. Cover plate and incubate for 15 minutes at room temperature.
11. Add 50 µL of stop solution to all wells.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Data Analysis

Calculation of Results

The Standard curve is constructed as follow:

1. Check prolactin standard values 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.
2. To construct the standard curve, plot the absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. 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	Conc.(ng/mL)	OD (450 nm)
1	0	0.07
2	3	0.19
3	6	0.36
4	25	0.79
5	100	1.45
6	200	2.01

Resource

Reference

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2. Duhau L, Grassi J, Grouselle D, Enjalbert A, Grognet JM. An enzyme immunoassay for rat prolactin: application to the determination of plasma levels.J Immunoassay. 1991; 12(2):233-50.
3. Gillet D, Ezan E, Ducancel F, Gaillard C, Ardouin T, Istin M, Menez A, Boulain JC, Grognet JM. Enzyme immunoassay using a rat prolactin-alkaline phosphatase recombinant tracer. Anal Chem. 1993 Jul 1; 65(13): 1779-84.
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5. Ferrag F, Lebrun JJ, Touraine P, Nagano M, Dardenne M, Kelly PA. Prolactin and the immune system. Immunomethods. 1994 Aug; 5(1):21-30.

Plate Layout

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