LSBio™ Rat PRL / Prolactin ELISA Kit

Catalog No. LS-F10462

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio

Rat Prolactin Antigen ELISA Kit

Catalog # LS-F10462 Strip well format. Reagents for up to 96 tests.

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www.lsbio.com

INTENDED USE

This rat prolactin antigen assay is intended for the quantitative determination of prolactin antigen in rat plasma. **For research use only.**

BACKGROUND

Rat prolactin (PRL) is a 197 aa, 23kD peptide hormone [1] that is secreted primarily by the pituitary gland in both males and females, though its major roles are in pregnancy and lactation [2,3]. Prolactin may have a role in breast cancer development, with higher prolactin levels correlating with postmenopausal breast cancer risk [4].

ASSAY PRINCIPLE

Rat prolactin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, anti-rat prolactin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with peroxidase conjugated secondary antibody. Following an additional washing step, TMB substrate is used for color development which is measured at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of rat prolactin. Color development is proportional to the concentration of prolactin in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-rat prolactin antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Rat prolactin standard:** 1 vial lyophilized standard
- **Anti-rat prolactin primary antibody:** 1 vial lyophilized antibody
- **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of rat prolactin standard:

Prolactin concentration (ng/ml)	Dilutions
1000	500µl (from vial)
500	500µl (BB) + 500µl (1000ng/ml)
200	600µl (BB) + 400µl (500ng/ml)
100	500µl (BB) + 500µl (200ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl Prolactin standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total rat prolactin in the 2-1000 ng/mL range. Samples with rat prolactin levels above 1000ng/mL should be diluted in blocking buffer before use. Normal plasma should not require dilution before use in this assay.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

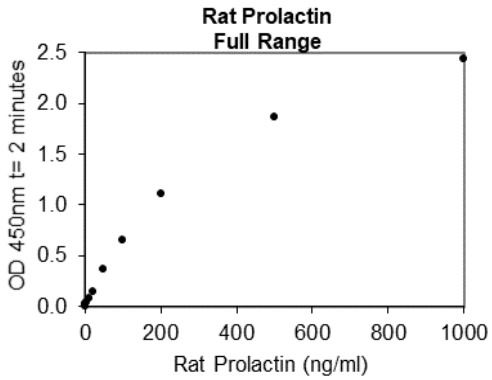
Measurement

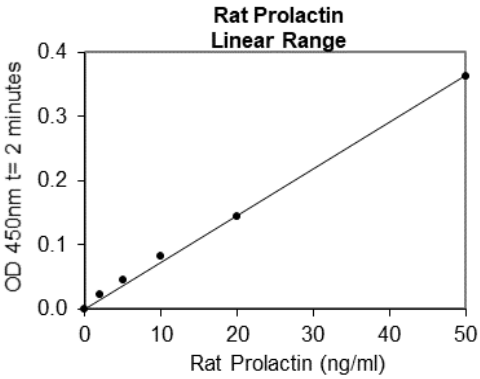
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of Prolactin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of Prolactin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

The concentration of prolactin in pooled normal rat plasma determined by in-house testing was 4.7-6.8 ng/ ml. The concentration in control rat plasma as determined by radioimmunoassay was 10.5 ng/ml [5].

Prolactin levels in pregnant rats are elevated immediately before birth [6].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.052-0.056) and calculating the corresponding concentration. The MDD was 0.36 ng/ mL.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Ellis S, *et al.*: Endocrinology. 1969, 85:886-894.
2. Goffin V, *et al.*: Annu Rev Physiol. 2002, 64:47-67.
3. Shiu RP, *et al.:* Annu Rev Physiol. 1980, 42:83-96.
4. Hankinson SE, *et al.*: J Natl Cancer Inst. 1999, 91:629-634.
5. Kaufman S, *et al.*: J Physiol. 1983, 336:73-81.
6. Jahn GA, *et al.*: J Reprod Fertil. 1986, 77:125-133.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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Technical.Support@LSBio.com