

Rat Prolactin ELISA Kit (Strip well format. Reagents for up to 96 tests.)

Catalog No. CS299A 1 kit
CS299B 5 kits

Intended Use: This rat prolactin antigen assay is intended for the quantitative determination of prolactin antigen in rat plasma.

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

Assay Principle: Rat prolactin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotinylated anti-rat prolactin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total prolactin present in the samples, is reacted with peroxidase-conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development which is measured at 450 nm. 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 microtiter strip plate (8 X12 removable wells):**
8 X12 well removable strips containing affinity purified anti-Rat prolactin antibody on the surface. Strips are blocked and dried.
- ◆**10X Wash Buffer:**
1 bottle of 50 ml; bring to 1X using DI water
- ◆**Rat prolactin standard**
1 vial of lyophilized standard
- ◆**Anti-Rat prolactin primary antibody**
1 vial of lyophilized biotinylated antibody
- ◆**Peroxidase conjugated streptavidin**
1 vial of concentrated streptavidin-HRP
- ◆**TMB substrate solution:**
1 bottle of 10 ml solution

Storage and Stability: All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.



Reagents and Equipment Required:

- 1-channel pipettes covering 0-10 μ l and 200-1000 μ l
- 12-channel pipette covering 30-300 μ l
- Paper towels or kimwipes
- 50 ml tubes, 1.5 ml centrifuge tubes
- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm.

Warnings:

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation Reagents:

- **TBS buffer:** 0.1 M Tris 0.15 M NaCl pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS
- **Wash buffer concentrate:** The wash buffer supplied in a 10x concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Collection:

The assay measures total Rat prolactin in the 0.1-100 ng/ml range. Samples with prolactin levels above 100ng/ml should be diluted in blocking buffer before use. Use of undiluted or 1:1 diluted normal plasma is suggested to ensure that resulting values fall within the linear range of the assay. Optimal dilutions should be experimentally determined by the researcher.

Assay Procedure:

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



Preparation of the Standard: Reconstitute standard as directed on the vial to give a 500 ng/ml solution.

Dilution table for preparation of rat prolactin standard:

Prolactin concentration (ng/ml)	Dilutions
100	800µl (BB) + 200µl (500ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2.5	500µl (BB) + 500µl (5ng/ml)
1	600µl (BB) + 400µl (2.5ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/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. Add 100 µl standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

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

Streptavidin-HRP Addition: Dilute 1 µl into 10 ml of blocking buffer and mix well. Add 2.5ml of the diluted secondary into 7.5 ml of blocking buffer (for a final dilution of 1:40,000) and add 100µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.



Substrate Incubation:

Add 100 μ l TMB substrate to all wells and shake plate for 4-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 μ l of 1 N H_2SO_4 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 and read final absorbance values at 450 nm. For best results read plate immediately

Measurement:

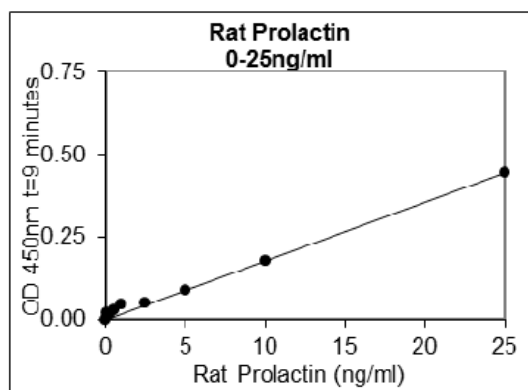
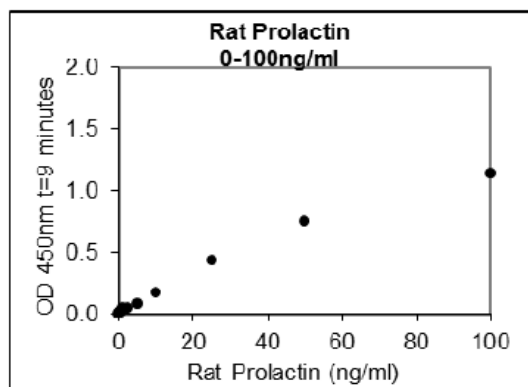
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:

Plot A_{450} against the amount of Rat prolactin in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total rat prolactin in the unknowns can be determined from this curve.

A typical standard curve.

(EXAMPLE ONLY, DO NOT USE)



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Expected Values:

The concentration of prolactin in pooled normal rat plasma determined by in-house testing was 6.8 ng/ml. The concentration in control rat plasma as determined by radioimmunoassay was 10.5 ng/ml. Prolactin levels in pregnant rats are elevated immediately before birth.

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 or from contact with the above product.

Example of ELISA Kit Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2.5ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	100ng/ml	
B	0	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2.5ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	100ng/ml	
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 22 Wells
Samples: 74 Wells

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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