

## Porcine prolactin Immunoassay

Catalog Number: SEKP-0022

For the quantitative determination of Porcine prolactin concentrations in cell culture supernates, serum, and plasma.

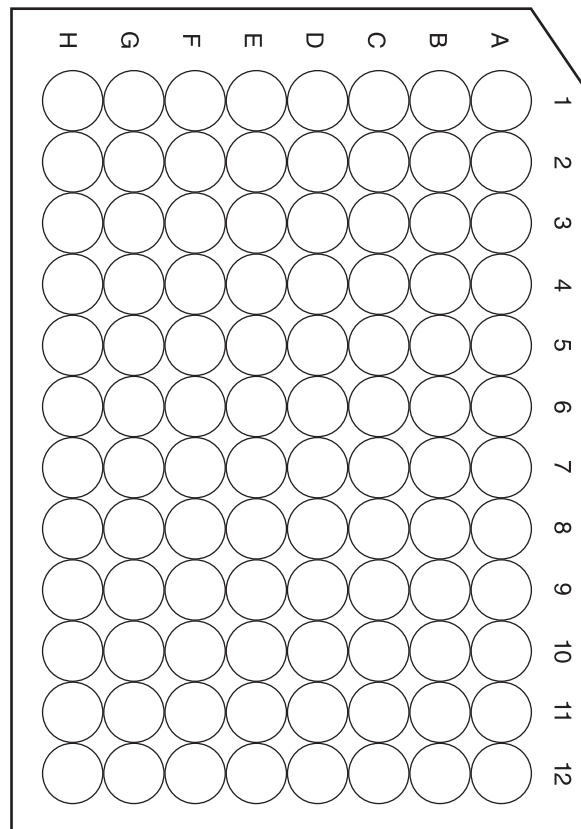
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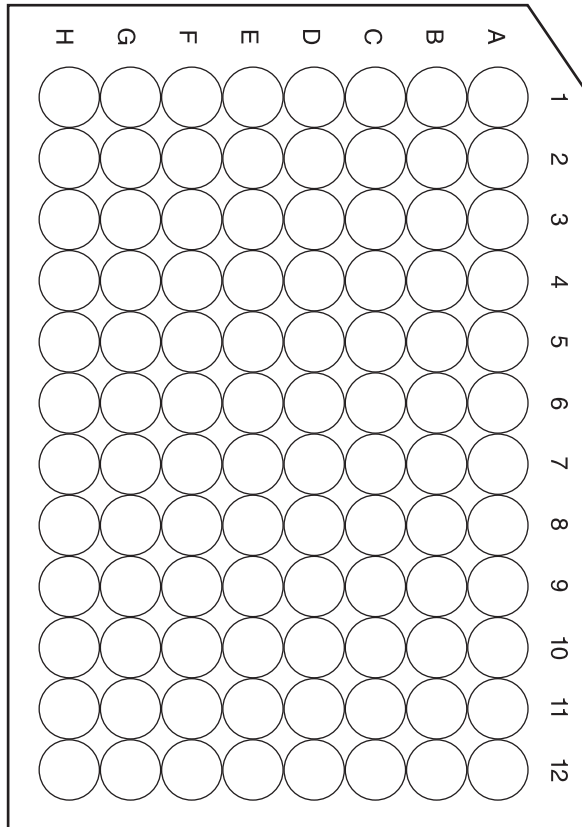
### MANUFACTURED AND DISTRIBUTED BY:

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd  
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.  
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

## TABLE OF CONTENTS

SECTION	PAGE
BACKGROUND.....	.01
PRINCIPLE OF THE ASSAY.....	.02
TECHNICAL HINTS AND LIMITATIONS.....	.02
PRECAUTIONS.....	.03
KIT COMPONENTS& STORAGE CONDITIONS.....	.04
OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED.....	.05
SPECIMEN COLLECTION & STORAGE.....	.05
REAGENTS PREPARATION.....	.05
ASSAY PROCEDURE.....	.07
CALCULATION OF RESULTS.....	.08
PERFORMANCE CHARACTERISTICS.....	.09
REFERENCES.....	.11



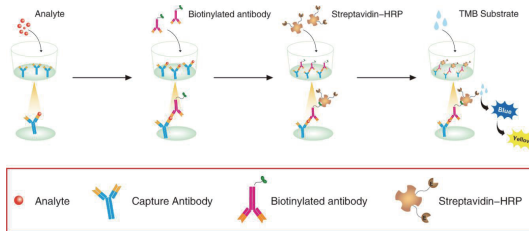


### BACKGROUND

Prolactin (PRL), also known as lactotrope, is a protein that in humans is encoded by the PRL gene. Although it is perhaps best known for its role in lactation, prolactin already existed in the oldest known vertebrates—fish—where its most important functions were probably related to control of water and salt balance. It stimulates the mammary glands to produce milk: Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands of the breasts and prepare for the production of milk. Prolactin also acts in a cytokine-like manner and as an important regulator of the immune system. Prolactin has important cell cycle related functions as a growth-, differentiating- and anti-apoptotic factor. As a growth factor binding to cytokine like receptors it has also profound influence on hematopoiesis, angiogenesis and is involved in the regulation of blood clotting through several pathways. More than 300 separate actions of PRL have been reported in various vertebrates. Prolactin acts in endocrine, autocrine, and paracrine manner through the prolactin receptor and a large number of cytokine receptors. Pituitary prolactin secretion is regulated by endocrine neurons in the hypothalamus, the most important ones being the neurosecretory tuberoinfundibulum (TIDA) neurons of the arcuate nucleus, which secrete dopamine to act on the D2 receptors of lactotrophs, causing inhibition of prolactin secretion. Many fishes have variants prolactin A and prolactin B. In humans 3 smaller (4, 16, and 22 kDa) and several larger variants exist. Highly elevated levels of prolactin decrease the levels of sex hormones — estrogen in women and testosterone in men. In humans, prolactin is produced at least in the pituitary, decidua, myometrium, breast, lymphocytes, leukocytes and prostate. Prolactin levels may be checked as part of a sex hormone workup, as elevated prolactin secretion can suppress the secretion of FSH and GnRH, leading to hypogonadism, and sometimes causing erectile dysfunction in men. Prolactin levels may be of some use in distinguishing epileptic seizures from psychogenic non-epileptic seizures. The serum prolactin level usually rises following an epileptic seizure.

**PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for prolactin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any prolactin present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for prolactin is added to detect the captured prolactin protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

**Schematic diagram:****TECHNICAL HINTS AND LIMITATIONS**

- 1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
- 2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
- 3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.

**REFERENCES**

- 1.Evans AM, et al. (1989). *Somat. Cell Mol. Genet.* 15 (3): 203–13.
2. Bole-Feysot C, et al. (1998). *Endocr. Rev.* 19 (3): 225–68.
3. Ben-Jonathan N, et al (1996). *Endocr. Rev.* 17 (6): 639–69.
4. Gerlo S, et al (2006). "Prolactin in man: a tale of two promoters". *Bioessays* 28 (10): 1051–5.
5. Banerjee S, et al. (2004). "Serum prolactin in seizure disorders". *Indian Pediatr* 41(8): 827–31.

## DESCRIPTION

**REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.

**RECOVERY:** The recovery of prolactin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

### Recovery of prolactin in two matrices

Sample Type	Average % of Expected	Range (%)
Citrate plasma	96	89-105
Cell culture supernatants	98	87-108

**LINEARITY:** To assess the linearity of the assay, three samples were spiked with high concentrations of prolactin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	95	102
	Range (%)	89-105	96-114
1:4	Average% of Expected	97	104
	Range (%)	90-104	97-116

## DESCRIPTION

4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

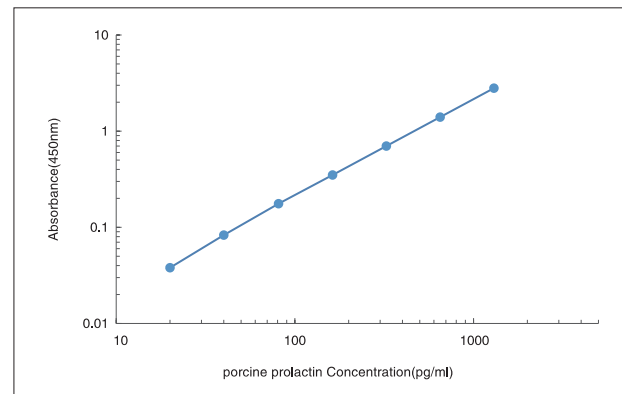
## PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## KIT COMPONENTS &amp; STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells x12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized, 2600 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vials	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C **for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C **for six months
Substrate Solution	1 bottle	Store at 2-8°C **for six months
Stop Solution	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

\*\*Provided this is within the expiration date of the kit.



Representative standard curve for prolactin ELISA.

## Performance Characteristics

**SENSITIVITY:** The minimum detectable dose was 5pg/mL.

**SPECIFICITY:** This assay recognizes both natural and recombinant Porcine prolactin. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

**ApoA1, BMP1, BMP2, BMP3, BMP4, HGF, HSP27, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFN $\gamma$ , MMP-2, MMP-9, PDGF-AA, PDGF-BB, PDGF-AB, IL2R, TGF $\beta$ 1, TGF $\beta$ 2, TGF $\beta$ 3, TLR1, TLR2, TLR3, TNF- $\alpha$ , TNF RI, TNF RII, VEGF.**

**CALCULATION OF RESULTS**

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the prolactin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the prolactin ELISA

Std (pg/mL)	O.D.1	O.D.2	Average	Corrected
0	0.015	0.018	0.016	---
20.3125	0.149	0.151	0.150	0.133
40.625	0.254	0.272	0.263	0.246
81.25	0.481	0.464	0.472	0.456
162.5	0.855	0.871	0.863	0.846
325	1.372	1.353	1.362	1.346
650	1.864	1.848	1.856	1.839
1300	2.55	2.57	2.560	2.543

**OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED**

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squir bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

**SPECIMEN COLLECTION & STORAGE**

**Cell Culture Supernates** - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8 C. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

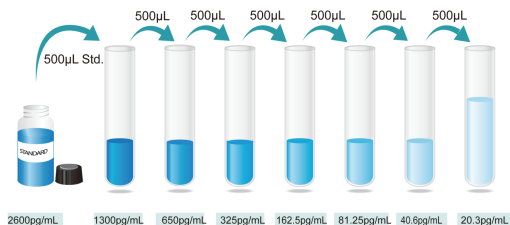
**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio**

**REAGENTS PREPARATION**

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25 C) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Sample(2 vials)** - Porcine prolactin Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard

curve. Reconstitute the Standard with 1.0mL of **Standard/Sample Diluent**. This reconstitution produces a stock solution of 2600pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 $\mu$ L of **Standard /Sample Diluent** into 1300pg/ml tube and the remaining tubes. Use the stock solution of 2600pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 1300pg/mL standard serves as the high standard. The **Standard /Sample Diluent** serves as the zero standard (0 pg/mL).



#### Preparation of Porcine prolactin standard dilutions

**\*If you do not run out of re-melting standard, store it at -20 $^{\circ}$ C. Diluted standard shall not be reused.**

4. Working solution of Biotin-Conjugate anti-Porcine prolactin antibody(1 vials) - The lyophilized Detection Antibody should be stored at 4 $^{\circ}$ C to -20 $^{\circ}$ C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110 $\mu$ L of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 50 $\mu$ L of detection antibody stock solution into 10mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial

antibody is used, make a 1:200 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

**\*The working solution should be used within one day after dilution.**

5. Working solution of Streptavidin-HRP(120 $\mu$ L) - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 $\mu$ L HRP Conjugate sufficient for a 96-well plate. Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100 $\mu$ L of HRP Conjugate to 10mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4 $^{\circ}$ C for up to 6 months. DO NOT FREEZE.

**\*The working solution should be used within one day after dilution.**

#### ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.



Add 100 $\mu$ l standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25 $\pm$ 2 $^{\circ}$ C).



Aspirate and wash 4 times

Add 100 $\mu$ l working solution of porcine-Conjugate anti-Porcine prolactin antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25 $\pm$ 2 $^{\circ}$ C).



Aspirate and wash 4 times

Add 100 $\mu$ l working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25 $\pm$ 2 $^{\circ}$ C).



Aspirate and wash 5 times

Add 100 $\mu$ l Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25 $\pm$ 2 $^{\circ}$ C). Protect from light.



Add 50 $\mu$ l Stop solution to each well. Read at 450nm within 5 minutes.