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Chicken prolactin Immunoassay

Catalog Number: SEKCN-0034
For the quantitative determination of Chicken prolactin concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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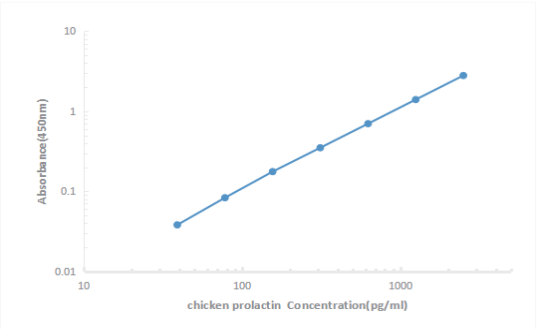
Recovery of prolactin in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	93	84-101
Cell culture supernatants	95	87-104

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

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	91	102
	Range (%)	83-101	95-113
1:4	Average% of Expected	94	105
	Range (%)	86-105	96-114



Representative standard curve for prolactin ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 10 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant Chicken 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.

ApoB, BMP1, EGF, IL-2, IFNγ, IGF-1, MMP2, PDGF, PIGF, TGFβ1, TLR1, TNF-α, VEGF-A

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.

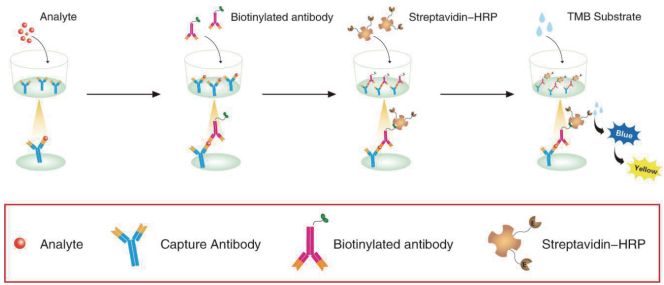
BACKGROUND

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 —

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.
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.

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	0.038	0.039	0.038	----
43.75	0.047	0.052	0.049	0.011
87.5	0.057	0.062	0.059	0.021
175	0.095	0.098	0.096	0.058
350	0.174	0.169	0.171	0.133
700	0.348	0.365	0.356	0.318
1400	0.925	0.916	0.920	0.882
2800	2.058	2.074	2.066	2.027

Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

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

Working solution of Streptavidin-HRP(120μL) - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120μL 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 ul of HRP Conjugate to 10 mL 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°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 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-Chicken prolactin antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature (25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

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& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 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℃**
Standard-lyophilized,2800pg/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
lyophilized Biotin-Conjugated antibody	1 vial	Store at 2-8°C **for six months
Concentrated Streptavi-din-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.

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.Squirt 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℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

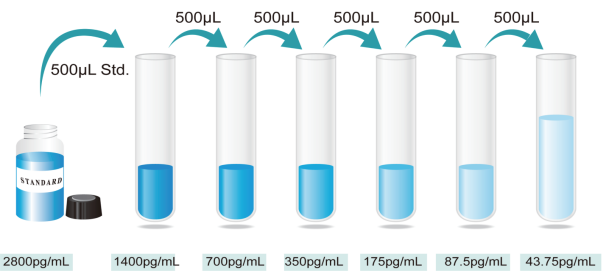
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: 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℃) 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)** - Chicken 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 2800 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.Pipette 500µL of Standard/Sample Diluent into 1400pg/ml tube and the remaining tubes. Use the stock solution of 2800pg/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 2800 pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0



Preparation of Chicken prolactin standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Chicken prolactin antibody(1 vial)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ 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 110µL 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 100µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection