

Prolactin ELISA



AL-199-i

INTENDED USE

Prolactin Enzyme-Linked Immunosorbent Assay (ELISA) Kit provides materials for the quantitative measurement of prolactin in human serum. This assay is intended for in vitro diagnostic use in the diagnosis and treatment of disorders of the anterior pituitary gland or of the hypothalamus portion of the brain.

SUMMARY AND EXPLANATION

Prolactin is a 198-amino acid, 23 kDa polypeptide hormone secreted in significant amounts by the anterior pituitary gland. The prolactin molecule has extensive sequence homology with growth hormone and placental lactogen¹. Recent studies have revealed molecular heterogeneity for prolactin in both pituitary extracts and blood^{2,3}. Prolactin secretion is regulated by complex mechanisms involving neurotransmitters and endocrine hormones. Many of the regulatory pathways involve hypothalamic secretion of dopamine, which inhibits prolactin secretion⁴. Prolactin, in synergy with estrogen, plays an important physiologic role in the initiation and maintenance of mammary gland growth and lactation in humans^{1,4}.

In addition, prolactin may have effects on cell growth in other tissues and on immune function. Particularly when present in high concentrations, prolactin may have inhibitory effects on gonadal function. Prolactin is present in several body fluids, including plasma, amniotic fluid, milk, mucosal secretions, and cerebrospinal fluid. Relative elevations in plasma prolactin concentrations occur during ovulation, pregnancy, nursing and stress^{1,2,4}. Abnormal elevations in plasma prolactin levels, or hyperprolactinemia, can occur as a result of pituitary adenomas, and may also be seen in other anatomic and traumatic abnormalities involving the pituitary gland (e.g., tumors, surgery, trauma), as a consequence of certain pharmacologic agents, and primary hypothyroidism. Low prolactin levels, or hypoprolactinemia, is observed in cases of hypopituitarism^{5,6}.

PRINCIPLE OF THE TEST

The Prolactin ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Prolactin antibody coated microtiter wells and incubated with assay buffer. After the first incubation, and washing, the wells are incubated with biotinylated Prolactin antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The biotin-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of Prolactin in the samples and calibrators.

MATERIALS SUPPLIED

CAL-199A Prolactin Calibrator A

One bottle, 2 mL, labeled Prolactin Cal. A, containing 0 ng/mL Prolactin in protein-based buffer and ProClin™ 300. Store unopened at 2-8°C until the expiration date.

CAL-199B - CAL-199F Prolactin Calibrators B – F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 1.4 - 244 ng/mL Prolactin in protein-based buffer and ProClin™ 300. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with **1 mL deionized water**. Solubilize, mix well and use after reconstitution. Aliquot and freeze in plastic vials immediately for multiple use and discard after the run. Avoid repeated freeze thaws.

Standardization Note: *The Prolactin calibrators are traceable to the World Health Organization International preparation NIBSC code 83/573 version 5.0.*

CTR-199-I & CTR-199-II Prolactin Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high Prolactin concentrations in protein-based buffer and ProClin™ 300. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with **1 mL deionized water**. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately in plastic vials for multiple use and discard after run. Avoid repeated freeze thaws.

PLT-199 Prolactin Antibody Coated Microtitration strips

One strip-holder, containing 12 strips and 96 microtitration wells with anti-Prolactin antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

ASB-199 Prolactin Assay Buffer

One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCR-199 Prolactin Biotin Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing biotinylated anti-Prolactin antibody in a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-199 Prolactin Streptavidin-Enzyme Conjugate-Ready-To-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2-30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate absorbance reader capable of absorbance measurement at 450 nm, 405 nm, and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Repeater Pipette

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

The following precautions should be observed:

- a) Follow good laboratory practice.
- b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
- c) Handle and dispose of all reagents and material in compliance with applicable regulations.

WARNING: Potential Biohazardous Material

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 6th Edition, 2020⁷.

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin™ 300 and Sodium azide⁸ as a preservative. ProClin™ 300 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the SDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION

- a) **Serum** is the recommended sample type.
- b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer’s instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.
- c) Samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.
- d) Avoid assaying lipemic, hemolyzed or icteric samples.
- e) Avoid repeated freezing and thawing of samples.
- f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Prolactin ELISA assay. It is the user’s responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **Prolactin Calibrators B-F and Prolactin Controls I & II:** Tap and reconstitute Prolactin Calibrators B-F and Prolactin Controls I & II each with 1 mL deionized water and solubilize for 10 minutes, vortex and use.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

1. Reconstitute Prolactin Calibrators B-F and Prolactin Controls I & II each with 1 mL deionized water as per the “Preparation of Reagents” section of this package insert. Solubilize for 10 minutes. Mix well by gentle vortex.
2. Label the microtitration strips to be used.
3. Pipette 20 µL of the **Calibrators, Controls, and unknowns** to the appropriate wells.
4. Add 100 µL of the **Prolactin Assay Buffer** to each well using a repeater pipette.
5. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
6. Aspirate and wash each strip **5 times (350 µL/per well)** with Wash Solution using an automatic microplate washer.
7. Add 100 µL of the **Prolactin Biotin Conjugate Ready-To-Use (RTU)** to each well using a repeater pipette.

8. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **20 minutes** at room temperature ($23 \pm 2^\circ\text{C}$).
9. Aspirate and wash each strip **5 times (350 μL /per well)** with **Wash Solution** using an automatic microplate washer.
10. Add **100 μL** of the **Streptavidin-Enzyme Conjugate-RTU** to each well using a repeater pipette.
11. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **15 minutes** at room temperature ($23 \pm 2^\circ\text{C}$).
12. Aspirate and wash each strip **5 times (350 μL /per well)** with the **Wash Solution** using an automatic microplate washer.
13. Add **100 μL** of the **TMB chromogen** solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
14. Incubate the wells, shaking at **600-800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature ($23 \pm 2^\circ\text{C}$).
NOTE: Visually monitor the color development to optimize the incubation time.
15. Add **100 μL** of the **Stopping Solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.

NOTE: Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.

- Prolactin ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Prolactin controls are printed on the **Calibration Card**.
- A full calibration curve, low, and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean Absorbance	Conc. (ng/mL)
A1, A2	Calibrators A	0.007 (Blank)	0
B1, B2	B	0.035	1.40
C1, C2	C	0.182	7.0
D1, D2	D	0.632	25.0
E1, E2	E	2.016	103.0
F1, F2	F	3.466	244.0

CAUTION: The above data must not be employed in lieu of data obtained by the user in the laboratory.

ANALYTICAL CHARACTERISTICS

Limit of Blank (LOB):
The Limit of Blank was 0.132 ng/mL, calculated from a minimum of n=18 measurements of analyte free sample.

Limit of Detection (LoD):
The lowest amount of Prolactin in a sample that can be detected with a 95% probability (n=14) is 0.4 ng/mL. The value was determined by processing six samples in the range of 1.5 to 5.0 ng/mL following CLSI EP17 guidelines. Eight assay runs were performed over four days with samples run in triplicates per run.

Limit of Quantification (LoQ):
The Limit of Quantification (LoQ) was 0.23 ng/mL, calculated from a minimum of n=18 measurements of calibrator and n=24 measurements of four samples in the range of 0.15-2.16 ng/mL.

Imprecision:
Based on NCCLS EP-10 guidelines reproducibility of the Prolactin ELISA assay was determined in a study using three samples. The study included a total of 6 assays, 3 replicates each, per assay (n=18). Representative data were calculated and are presented in the following table.

Sample	Mean conc. (ng/mL)	Repeatability		Intermediate Precision	
		SD	%CV	SD	%CV
Pool-1	7.131	0.198	2.78%	0.240	3.37%
Pool-2	30.206	0.645	2.13%	0.906	3.00%
Pool-3	103.551	3.582	3.46%	7.800	7.53%

Linearity:
Based on NCCLS EP-6-P multiple dilutions of the three serum samples containing various Prolactin levels were diluted with Calibrator A. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
Sample 1	Neat Value	64.299		111%
	2	32.150	35.255	

RESULTS

NOTE: The results in this package insert were calculated by plotting the log optical density (OD) data on the y-axis and log Prolactin concentration on X-axis using a cubic regression curve fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Optimum results can be obtained at incubation temperature of ($23 \pm 2^\circ\text{C}$).
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Prolactin concentrations in ng/mL along the x-axis, using a cubic regression curve fit.
4. Determine the Prolactin concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Prolactin concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately further diluted with the 0 ng/mL (Cal. A) and re-assayed.
6. Any sample reading lower than the LoD should be reported as such.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Prolactin levels in mouse and human EDTA plasma. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.⁹

As with all diagnostic tests, a definite clinical diagnosis **should NOT** be based on the results of a **single test**. The diagnosis should only be made by the physician after reviewing all clinical and laboratory findings.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to ensure proper performance.

	4	16.075	17.431	
	8	8.037	9.096	
	16	4.019	4.506	
Sample 2	Neat Value	58.410		111%
	2	29.205	30.698	
	4	14.603	16.361	
	8	7.301	8.459	
Sample 3	Neat Value	31.911		111%
	2	15.956	17.223	
	4	7.978	9.155	

Recovery:

Known amounts of Prolactin were added to five serum samples containing different levels of endogenous Prolactin. The concentration of Prolactin was determined before and after the addition of exogenous Prolactin and the percent recovery was calculated.

Sample	Endogenous Conc. (ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
S1	4.692	17.657	16.716	100%
		28.223	27.093	95%
		40.788	37.617	93%
S2	3.166	15.208	16.589	109%
		27.249	26.121	96%
		39.291	39.301	100%
S3	9.288	21.024	20.333	97%
		32.759	30.274	92%
		44.495	39.774	89%
S4	53.112	62.656	59.917	96%
		72.201	68.460	95%
		81.745	78.007	95%
S5	13.176	24.717	23.452	95%
		36.258	33.987	94%
		47.800	38.904	81%

Analytical Specificity:

Cross-Reactivity

FSH, LH, hCG, and TSH when tested at twice the physiological dose were below the detection limit of the assay. Monoclonal antibody pair used in the assay detects human, bovine and monkey Prolactin.

Hook Effect:

There is no high-dose hook effect at Prolactin concentrations up to 3000 ng/mL.

Expected Value:

The expected prolactin concentration ranges (95% CI) for Prolactin in males and females were calculated and listed in the table below.

Population	Age Range (Yrs.)	No. of Specimens	Median Prolactin Conc. (ng/mL)	Prolactin Range (ng/mL)
Male	18 - 89	50	20.8	4.9 - 58.2
Female	15 - 91	54	25.6	6.3 - 236.9

The expected prolactin concentration ranges (95% CI) for Prolactin in pregnant females in first and second trimesters were calculated and listed in the table below.

Population	Age Range (Yrs.)	Gest. Age (wks., days)	No. of Specimens	Median Prolactin Conc. (ng/mL)	Prolactin Range (ng/mL)
1st Trimester	17 - 40	11,5 - 14,1	19	52.9	31.9 - 256.1
2nd Trimester	19 - 42	15,2 - 20,1	20	105.8	37.1 - 237.6

Interference:

When potential interferents (Hemoglobin, biotin, bilirubin, and intralipids) were added at least two times their physiological concentration to control sample, Prolactin concentration were within ± 10% of the control as represented in the following table. This study was based on NCCLS EP-7.

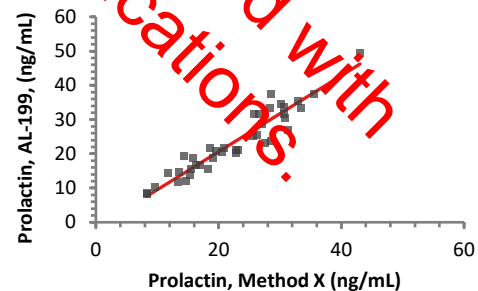
Interferents	Interferent Dose	Analyte Conc. (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1 mg/mL	12.24	11.86	-3.1
	0.5 mg/mL	13.03	12.78	-1.9
	0.1 mg/mL	12.40	12.40	0.0
Hemoglobin	1 mg/mL	15.50	15.32	-1.2
	0.5 mg/mL	16.59	16.65	0.4
	0.1 mg/mL	17.54	16.81	-4.1
Biotin	1200 ng/mL	12.97	12.53	-3.4
	600 ng/mL	12.85	12.95	0.8
	200 ng/mL	12.57	12.91	2.7
Biotin	1200 ng/mL	15.78	15.74	-0.3
	600 ng/mL	16.41	16.93	3.1
	200 ng/mL	17.63	18.21	3.3
Intralipids	20 mg/mL	18.11	18.52	2.3
	10 mg/mL	18.54	18.52	-0.1
	5 mg/mL	18.72	18.67	-0.2
Intralipids	20 mg/mL	21.59	22.53	4.3
	10 mg/mL	24.12	24.80	2.8
	5 mg/mL	25.28	23.56	-6.8
Bilirubin	0.66 mg/mL	13.87	13.52	-2.6
	0.2 mg/mL	16.82	17.23	2.4
Bilirubin	0.66 mg/mL	15.31	15.56	1.7
Bilirubin	0.2 mg/mL	20.97	21.81	4.0

Method Comparison:

The Prolactin ELISA (AL-199) has been compared to 40 samples obtained from a commercial vendor (Method X).

Passing statistical analysis of the results yielded the following Regression:

$$\text{Prolactin (AL-199)} = -1.612 (\text{ng/mL}) + 1.111 (\text{Method X}), (R=0.947)$$



REFERENCES

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






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Symbols used with Ansh Labs Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
	Distributed by	Distributor	Distributeur	Distribuidor	Distributore
	Content	Inhalt	Conditionnement	Contenido	Contenuto
	Volume/No.	Volumen / No.	Volumen/Quantité	Volumen/Número	Volumen/Quantità

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