ab108679 – Prolactin ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of Prolactin in Human Serum.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's Prolactin *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Prolactin in Human serum.

A 96-well plate has been pre-coated with Streptavidin. Samples and the Prolactin-HRP conjugate are added to the wells, where Prolactin in the sample competes with the added Prolactin-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Prolactin in the sample and the intensity is measured at 450 nm.

Prolactin is a polypeptide hormone synthesised and secreted by the Adenohypophysis (anterior Pituritary gland) and the placenta. It is also produced in other tissues including the breast and the decidua. Pituritary Prolactin secretion is regulated by neuroendrocrine neurons in the hypothalamus, most importantly by neurosecretory dopamine neurons of the arcuate nucleus, which inhibit Prolactin secretion.

Prolactin is present in several body fluids, including blood plasma, amniotic fluid, milk, mucosal secretions and cerebrospinal fluid. Prolactin has many effects, the most important of which is to stimulate the mammary glands to produce milk (lactation). Other possible functions of Prolactin include the surfactant synthesis of the fetal lungs at the end of the pregnancy and immune tolerance of the foetus by the maternal organism during pregnancy.

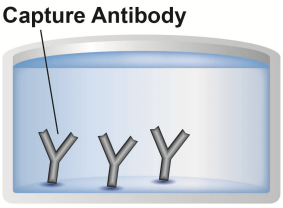
Prolactin may also have inhibitory effects on gonadal function when present in high concentrations.

There is a diurnal cycle in Prolactin secretion. During pregnancy, high circulating concentrations of estrogen promote Prolactin production. The resulting high levels of Prolactin secretion cause maturation of the mammary glands, preparing them for lactation. After childbirth, Prolactin levels fall as the internal stimulus for them is removed.

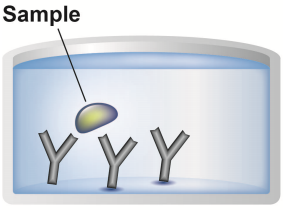
High Prolactin levels also tend to suppress the ovulatory cycle by inhibiting the secretion of both FSH and GnRH.

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. Elevations in plasma Prolactin concentrations occur during ovulation, pregnancy, nursing and stress. Abnormal elevations in plasma Prolactin levels (hyperprolactinemia) can occur as a result of pituitary adenomas, other anatomic and traumatic abnormalities, in response to certain pharmacologic agents and in hypothyroidism. Hypoprolactinemia (low Prolactin levels) are observed in cases of hypopituitarism.

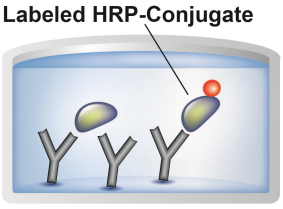
2. ASSAY SUMMARY



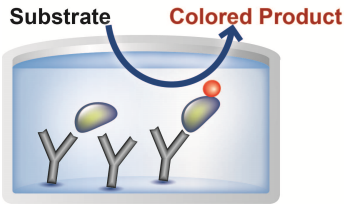
Prepare all reagents, samples, controls and standards as instructed.



Add samples, standards and controls to wells used.



Add prepared labeled HRP-Conjugate to each well. Incubate at 37ºC.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Streptavidin Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
Anti-Prolactin-HRP Conjugate	12 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
10X Wash solution	50 mL	2-8°C
Prolactin Control	1 mL	2-8°C
Prolactin Standard 0 – 0 ng/mL	1 mL	2-8°C
Prolactin Standard 1 – 5 ng/mL	1 mL	2-8°C
Prolactin Standard 2 – 10 ng/mL	1 mL	2-8°C
Prolactin Standard 3 – 25 ng/mL	1 mL	2-8°C
Prolactin Standard 4 – 50 ng/mL	1 mL	2-8°C
Prolactin Standard 5 – 100 ng/mL	1 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 1X Wash Solution

Dilute contents of wash solution concentrate 10X to 500 mL with distilled water in a suitable storage container. For smaller volumes respect the 1:10 ratio. The diluted wash solution is stable for 30 days at 2 - 8°C.

It is possible to observe crystals in the concentrated wash solution. In this case mix at room temperature until complete dissolution of crystals; for greater accuracy dilute the whole content of the bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely, then mix until crystals are completely dissolved.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- The determination of Prolactin can be performed in Human serum. Microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay. If the assay is performed on the same day of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 min. before testing. For samples with concentration above 100 ng/ml dilute 1:2 with Standard 0.

  Avoid repeated freezing and thawing

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 50 µL standards, control and samples into their respective wells. Add 100 µL 1X Prolactin-HRP Conjugate to each well. Leave a blank well for substrate blank.

13.4. Cover wells with the foil supplied in the kit.

13.5. Incubate for 1 hour at 22 - 28°C.

13.6. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL distilled water. Avoid overflows from the reaction wells. The soak time between each wash cycle should be > 5 seconds. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step.

13.7. Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.

13.8. Add 100 µL TMB Substrate Solution into all wells.

13.9. Incubate for exactly 15 minutes at room temperature in the dark.

13.10. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

13.11. Measure the absorbance of the sample at 450 nm within 30 minutes of addition of the Stop Solution.

13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

14. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

Each laboratory must establish its own normal ranges based on patient population. The serum or plasma Prolactin values are comprised in the following intervals:

	Range (ng/mL)
Male	1.8 - 17.0
Female: Menstrual Cycle	1.2 - 19.5
Female: Menopause	1.5 - 18.5

Some female of the population tested in this group were probably using oral contraceptives, which may affect results.

SENSITIVITY –

The lowest detectable concentration of Prolactin that can be distinguished from the zero standard is 0.12 ng/mL at the 95 % confidence limit.

PRECISION –

Intra Assay Variation:

Within-run precision was determined by replicate determinations (20x) of three different control sera in one assay.

Sample	N	X	δ	C.V.%
Level 1	20	5.33	0.15	2.78
Level 2	20	18.212	0.73	4.03
Level 3	20	37.20	1.38	3.71

Inter Assay Variation:

Between-run precision was determined by replicate measurements (10x) of three different control sera with kits of different lots.

Sample	N	X	δ	C.V.%
Level 1	10	5.46	0.30	5.49
Level 2	10	17.72	0.91	5.16
Level 3	10	36.29	1.67	4.60

RECOVERY –

The recovery of 3.13 – 6.25 – 12.50 – 25.00 – 50.00 ng/ml of Prolactin added to sample gave an average value (±SD) of 102.52% ± 9.75% with reference to the original concentrations.

The dilution test performed on three sera diluted 2 - 4 - 8 - 16 times gave an average value (±SD) of 102.19% ± 9.80%.

15. ASSAY SPECIFICITY

The cross reaction of the antibody calculated at 50 % is:

Human Prolactin	100 %
Luteinizing Hormone	None Determined
Follicle-stimulating Hormone	None Determined
Human Chorionic Gonadotropin	None Determined
Thyroid-stimulating hormone	None Determined
Human Growth Hormone	None Determined

The method allows the determination of Prolactin from 5 – 100 ng/mL.

16. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

17. NOTES

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