AccuDiag™ Prolactin ELISA Kit

REF 4226-16

IVD See External Label 2°C 8°C Σ 96 Tests

Prolactin ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-200 ng/ml
Sample	50 µL Serum
Specificity	96%
Sensitivity	2.0 ng/ml
Incubation Time	80 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

For the quantitative determination of prolactin concentration in human serum.

SIGNIFICANCE AND SUMMARY

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and woman. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor. Women normally have slightly higher basal prolactin levels than men; apparently, there is an estrogen-related rise at puberty and a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation. Prolactin also suppresses gonadal function. During pregnancy, prolactin levels increase progressively to between 10- and 20-times normal values, declining to non-pregnant levels by 3-4 weeks post-partum. Breast-feeding mothers maintain high levels of prolactin, and it may take several months for serum concentrations to return to non-pregnant levels. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High prolactin levels are commonly associated with galactorrhea and amenorrhea. Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanism. Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of prolactin is episodic and demonstrates diurnal variation. Mildly elevated prolactin concentrations should be evaluated taking these considerations into account. Prolactin concentrations may also be increased by drugs such as chloropromazine and reserpine, and may be lowered by bromocyptine and L-dopa.

ASSAY PRINCIPLE

The Prolactin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

REAGENTS

Materials provided with the test kit

1. Antibody-coated microtiter wells
2. Reference standard set, contains 0, 5, 20, 50, 100, and 200 ng/ml, human prolactin, in liquid form (ready to use) or lyophilized form.
3. Enzyme Conjugate Reagent, 12 ml.
4. TMB Substrate, 12 ml.
5. Stop Solution, 12 ml.
6. Wash Buffer Concentrate(50X), 15ml
7. Control Set (Optional)

Materials required but not provided

1. Precision pipettes: 40µl~ 200µl, and 1.0ml.
2. Disposable pipette tips.
3. Distilled water.
4. Vortex mixer or equivalent.

5. Absorbent paper or paper towel.
6. Graph paper.
7. Microtiter well reader.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) before use.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into sink.
7. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB substrate into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 5 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter well reader within 15 minutes.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

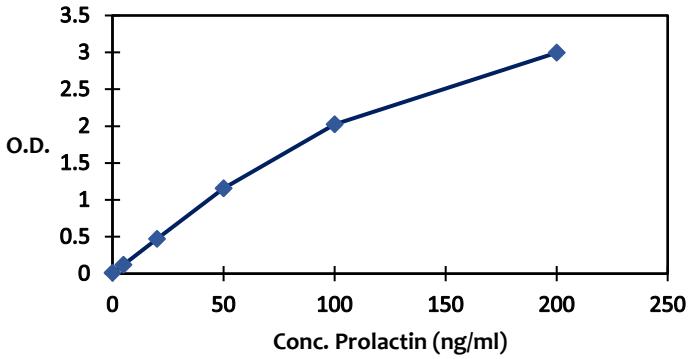
RESULTS

Calculate the mean absorbance value (A_{450}) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of prolactin in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of typical standard run with optical density reading at 450nm shown in the Y axis against Prolactin concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Prolactin (ng/ml)	Absorbance (450nm)
0	0.010
5	0.121
20	0.472
50	1.158
100	2.022
200	2.995



EXPECTED VALUES AND SENSITIVITY

Each laboratory must establish its own normal ranges based on patient population. Based on a limited number of healthy adult blood specimens, the mean prolactin concentrations in males (N=90) and females (N=120) are estimated to be 6 and 15 ng/ml, respectively. The minimal detectable concentration of human prolactin by this assay is estimated to be 2 ng/ml.

STORAGE

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2.5 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REFERENCES

1. Uotila M., Ruouslahti E. and Engvall E. **J. Immunol. Methods** 1981; 42:11-15
2. Shome B. and Parlow A.F. **J. Clin. Endocrinol. Metab.** 1977;45:1112-1115
3. Cowden E.A., Ratcliffe W.A., Beastall G.H. and Ratcliffe J.G.**Annals Clin. Biochem** 1979; 16: 113-121
4. Frantz A.G. **N. Engl. J. Med.** 1978; 298: 201-207
5. Jacobs L., Snyder P., Wilber J., Utiger R. and Daughaday W.J. **Clin. Endocrin.** 1978; 33: 99

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016 ISO 13485 Quality Management for Medical Devices CERTIFIED Diagnostic Automation/Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA	
Date Adopted	2022-09
REF 4226-16	AccuDiag™ - Prolactin ELISA
Brand Name	AccuDiag™
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu
Revision Date: 2017-09	