Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

PROLACTIN Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAPRL1	Prolactin Elisa	96 Tests

Intended Use:

Prolactin Elisa is intended to be used for the quantitative determination of Prolactin in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Prolactin is synthesized in the anterior pituitary and is secreted in episodes. The hormone is made up of 198 amino acids and has a molecular weight of approx. 22-23 kD. Prolactin appears in serum in three different forms. The biologically and immunologically active monomeric ("little") form predominates (approx. 80 %), 5-20 % is present as the biologically inactive dimeric ("big") form and 0.5-5 % is present as the tetrameric ("big-big") form having low biological activity. The target organ for prolactin is the mammary gland, the development and differentiation of which is promoted by this hormone. High concentrations of prolactin have an inhibiting action on steroidogenesis of the ovaries and on hypophyseal gonadotropin production and secretion. During pregnancy the concentration of prolactin rises under the influence of elevated estrogen and progesterone production. The stimulating action of prolactin on the mammary gland leads post-partum to lactation. Hyperprolactinemia (in men and women) is the main cause of fertility disorders. The determination of prolactin is utilized in the diagnosis of anovular cycles, hyperprolactinemic amenorrhea and galactorrhea, gynecomastia and azoo-spermia. Prolactin is also determined when breast cancer and pituitary tumors are suspected.

Sandwich principle. Total duration of assay: **80 minutes.**

- Sample, Anti-Prolactin coated microwells and enzyme labelled Anti-Prolactin are combined.
- During the incubation, prolactin presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the prolactin within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of PRL in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with mouse monoclonal Anti-Prolactin. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Stable for 2 months at the above storage condition.
Prolactin Calibrators	6x1ml	6 vials containing Prolactin at concentrations of 0.0, 5, 10, 20, 50 and 100 ng/ml made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once opened the material is stable for a period of one month when stored tightly capped and without contamination at 2-8°C.
Enzymatic Conjugate	1x11ml	1 vial containing 11ml of HRP labelled mouse monoclonal Anti-Prolactin in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300® preservative. Once opened the material is stable for a period of one month when stored tightly capped and without contamination at 2-8°C.
Wash Buffer Concentrate (40X)	1x25ml	PBS-Tween wash solution. 40X concentrate. Prepare Wash Buffer by diluting the Wash Buffer Concentrate with 975ml of distilled water. Once diluted the wash buffer is stable for 2 months when stored at 15 – 25°C.
Substrate Solution	1x11ml	Ready to use, tetramethylbenzidine TMB. Once opened the material is stable for a period of two months when stored tightly capped and without contamination at 2-8°C.
Stop Solution	1x6ml	1 vial, 6ml of 1mol/L sulfuric acid. Once opened the material is stable for a period of two months when stored tightly capped and without contamination at 2-8°C.

IFU and Cardboard plate covers.

Materials required but not provided:

Microplate reader with 450nm and 620nm wavelength absorbent capacity, microplate washer, incubator, plate shaker, micropipettes and multichannel micropipettes delivering 50µ with a precision of better than 1.5%, absorbent paper, distilled water.

Precautions and warnings:

- For in vitro diagnostic use only. For professional use only.
- All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all products should be reared as potential biohazards in use and for disposal.
- Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
- Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
- Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Do not use reagents beyond the labelled expiry date.
- Do not mix or use components from kits with different batch codes.
- If more than one plate is used, it is recommended to repeat the calibration curve.
- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Ensure that the bottom of the plate is clean and dry.
- Ensure that no bubbles are present on the surface of the liquid before reading the plate.
- The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

Specimen Collection:

1. Collect serum samples in accordance with correct medical practices.
2. Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8 °C, and 1 month at -20 °C. Recovery within 90-110 % of serum value or slope 0.9-1.1.

Freeze only once.

Storage and Stability:

Store at 2-8°C. Place unused wells in the zip-lock aluminium foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return unused calibrators to 2-8 °C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles. Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
- Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
- Avoid grossly hemolytic, lipemic or turbid samples.
- Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
- Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
- Adjust the incubator to 37 °C.
- Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
- Don's use Substrate if it looks blue.
- Don't use reagents that are contaminated or have bacteria growth.

Quality Control:

Each laboratory should establish assay controls at levels in the low, normal, and elevated range for monitoring assay performance. There controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Procedure:

STEP 1

Preparation: Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.

STEP 2

Addition of Calibrators: Add 25µl of calibrators or samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100µl of the Enzyme Conjugate solution to each well. Shake the plate for 30 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 5

Washing: Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper. Add 350µl if wash solution, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

Addition of the Substrate: Add 100µl of Substrate Solution to each well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark. Do not shake the plate after substrate addition.

STEP 8

Stopping the Reaction: Add 50µl of the Stop solution into each well. Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue colour changes to yellow completely.

STEP 9

Measurement: Read the absorbance of the wells at 450 (using 620 to 630nm as the reference wavelength to minimize well imperfections). The results should be read within 30 minutes of adding the stop solution.

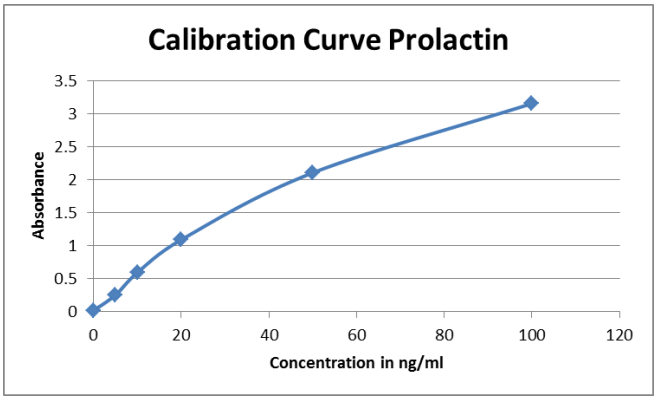
Calculation of results:

- Record the absorbances obtained from the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in ng/ml for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Example:

ID	ABSORBANCE	Value
CAL A	0.015	0.0 ng/ml
CAL B	0.183	5.0 ng/ml
CAL C	0.492	10.0 ng/ml
CAL D	1.153	20.0 ng/ml
CAL E	2.107	50.0 ng/ml
CAL F	3.158	100.0 ng/ml
Control 1	0.23	5.84 ng/ml
Control 2	1.11	28.71 ng/ml
Sample	1.76	48.96 ng/ml



DO NOT USE THE ABOVE CURVE OR VALUES IN LIEU OF THE CALIBRATION. CALIBRATION CURVES DIFFER DUE TO INSTRUMENT USED AND TECHNIQUE ADOPTED.

Limitations - interference:

•The assay is unaffected by icterus (bilirubin < 513 µmol/l or < 30 mg/dl), hemolysis (Hb < 0.932 mmol/l or < 1.5 g/dl), lipemia (Intralipid < 1500 mg/dl) and biotin (< 164 nmol/l or < 40 ng/ml).
• Criterion: Recovery within ± 15 % of initial value.
• Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
• No interference was observed from rheumatoid factors up to a concentration of approx. 1100 IU/mL.
• There is no high-dose hook effect at prolactin concentrations up to 220000 µIU/mL (10000 ng/mL).
• In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.
• When determining prolactin it should be remembered that the measured concentration is dependent upon when the blood sample was taken, since the secretion of prolactin occurs in episodes and is also subject to a 24-hour cycle.
• The release of prolactin is promoted physiologically by suckling and stress. In addition, elevated serum prolactin concentrations are caused by a number of pharmaceuticals (e.g. dibenzodiazepines, phenothiazine), TRH and estrogen.
• The release of prolactin is inhibited by dopamine, L-dopa and ergotamine derivatives.
• A number of publications report the presence of macroprolactin in the serum of female patients with various endocrinological diseases or during pregnancy. Differing degrees of detection of the serum macroprolactins relative to monomeric prolactin (22-23 kD) by various immunoassays have also been described. This could make the detection of hyperprolactinemia dependent on the immunoassay used.
• In case of implausible high prolactin values a precipitation by polyethylene glycol (PEG) is recommended in order to estimate the amount of the biological active monomeric prolactin.
• For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges:

Measuring range

1.00-2128 µIU/mL or 0.0470-100 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 1 µIU/mL or < 0.0470 ng/mL. Values above the measuring range are reported as > 2128 µIU/mL or > 100 ng/mL.

Lower limits of measurement

Lower detection limit

Lower detection limit: 1.00 µIU/mL (0.047 ng/mL)

The lower detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 20).

Dilution

Samples with prolactin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10. The concentration of the diluted sample must be > 50 µIU/mL or > 2.4 ng/mL concentration.

Expected values

Men: 2.3 - 17.5 ng/mL
Women: 2.9 - 25.8 ng/mL

A study with the Prolactin II assay was performed using samples from 300 apparently healthy blood donors. The following results were obtained.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

		Percentiles			
		50th	2.5-97.5th	50th	2.5-97.5th
	N	µIU/mL		ng/mL	
Men	404	189	74-350	8.91	3.5-16.5
Women (not-pregnant)	1169	268	84-605	12.64	4-28.5

Performance Characteristics:

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human Serum 1	4.73	0.409	8.64	0.411	8.68
Human Serum 2	16.84	1.206	7.16	1.295	7.69
Human Serum 3	42.74	2.167	5.07	2.334	5.46
PC Universal 1	8.76	0.617	7.04	0.686	7.83
PC Universal 2	17.86	1.047	5.86	0.943	5.28

*Repeatability = within-run precision

Method comparison

A comparison of the Prolactin assay (y) with the Roche Cobas Prolactin II (x) using clinical samples gave the following correlations: Number of samples measured: 121
Linear regression
y = 1.0421x + 0.048
r = 0.9863
The sample concentrations were between approx. 0 and 121 ng/mL.

Analytical specificity

The monoclonal antibodies used are highly specific against prolactin. No cross reaction with hGH, hCG, hPL, TSH, FSH and LH has been observed.

References

1. Smith CR, Norman MR. Prolactin and growth hormone: molecular heterogeneity and measurement in serum. Ann Clin Biochem, 1990; 27: 542-550.
2. Runnebaum B, Rabe T. Gynäkologische Endokrinologie und Fortpflanzungsmedizin Springer Verlag 1994. Band 1:21,124-126,179-181,613, Band 2:412-417,436. ISBN 3-540-57345-3, ISBN 3-540-57347-X.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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