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 oestrogen 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 tumours 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 present in the sample reacts simultaneously with the two antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies.
• Substrate solution is added for which the colorimetric biochemical reaction is catalysed by the label on the complexes resulting in a colour development which is measured spectrophotometrically.
• The absorbance is proportional to the amount of Prolactin 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 2-8ºC. Stable for 2 months at 2-8ºC. |
| Prolactin Calibrators | 6x1ml | 6 vials containing Prolactin (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 2-8ºC. |
| Enzymatic Conjugate | 1x11ml | 1 vial containing 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 2-8ºC. |
| 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 – 25ºC. |
| 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 2-8ºC. |
| Stop Solution | 1x6ml | 1 vial sulfuric acid (1 mol/l). Once opened the material is stable for two months when stored tightly capped and without contamination at 2-8ºC. |

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, 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. However, since no analytical method that can fully assure that infectious agents in the specimens or reagents are completely absent, all reagents should be handled 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 poor ambient conditions such as air containing high concentrations of corrosive vapours including sodium hypochlorite acid, alkalis and acetaldehydes or containing dust.
• Wash the wells thoroughly Each well must be filled with wash solution but avoid overflowing which may contaminate adjacent wells. After each wash cycle fully expel the liquid with a sharp flick of the plate. Tap the microplate onto absorbent paper to remove all residual wash solution after the final wash step. Washing the microplate with an automated microplate strip washer is recommended. Failure to adhere to these washing guidelines may result in poor replicate well consistency and spurious results.
• Do not use reagents beyond the labelled expiry date.
• Do not mix or use components from kits of different batch number.
• 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:**
Collect serum samples by separation from red blood cells after standard venepuncture technique. Store the serum at 15 - 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 2-8ºC. 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 brought to ambient temperature (15-25 °C) before use.
• 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 which should be sufficient to remove particulate matter.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (15 -25 °C) before measurement.
• Mix all reagents through gently inverting prior to use.
• Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
• Don's use Substrate if the reagent is blue in colour instead of colourless.
• Don't use reagents that are contaminated or have bacteria growth.

**Quality Control:**
Each laboratory should establish assay controls corresponding to low, normal, and elevated levels of the normal reference range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. Results for unknown samples within a plate are valid if the control values fall within assigned concentration ranges.

**Procedure:**

**STEP 1**
**Preparation:** Use only the number of wells required and assign wells for each calibrator, control and sample to be assayed.

**STEP 2**
**Addition of Sample:** Add 25µl of calibrators, controls 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 37ºC.

**STEP 5**
**Washing:** Discard the contents of the micro plate by decantation or aspiration. If decanting, flick the plate sharply upside down. Add 350µl if wash solution, decant (flick to expel wash solution) 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 approximately 20 seconds to mix the liquid within the wells. It is important to ensure that the blue colour changes to yellow completely.

**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.

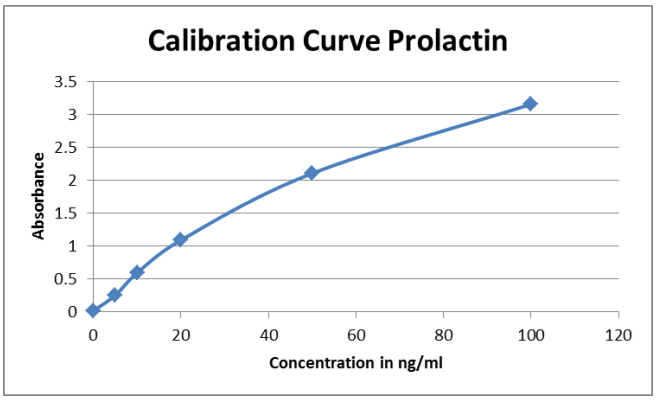
## Calculation of results:

- 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), haemolysis (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 oestrogen.
• 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. Different immunoassays show differing degrees of detection of serum macroprolactin relative to monomeric prolactin (22-23 kD) so the detection of hyperprolactinemia is dependent on the immunoassay used.
• Where implausibly high prolactin values are obtained, perform precipitation by polyethylene glycol (PEG) is recommended to confirm presence of macroprolactin and to estimate the amount of the biological active monomeric prolactin present.
• Diagnosis of conditions involving prolactin should not be made solely on the result of this ELISA, but 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 - 2128 µIU/mL or 0.047 - 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 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 phosphate buffered saline. 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): twice 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 = 1.042x + 0.048
r = 0.986
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.

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|---|---|---|---|
| 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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