Atlas Medical CE

# PROLACTIN ELISA

## Enzyme Immunoassay for the Quantitative Determination of Prolactin Concentration in Human Serum

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C **Store at 2-8 °C**

Σ **96 Tests**

**Intended use**

Immunoassay for the in vitro quantitative determination of prolactin in human serum.

**Introduction**

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 azoospermia. Prolactin is also determined when breast cancer and pituitary tumors are suspected.

**Test Principle**

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

- Sample, Anti-Prolactin coated microwells and enzyme labeled 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.

**Materials**

**Materials provided**

1. **Coated Microplate**, 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-Prolactin.
2. **Calibrators**, 6 white cap vials, 1 ml each, ready to use; Concentrations: 0(A), 5(B), 10(C), 20(D), 50(E) and 100(F) ng/mL.
3. **Enzyme Conjugate**, 1 red cap vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-prolactin in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300® preservative.
4. **Substrate**, 1 brown cap vial, 11ml, ready to use, (tetramethylbenzidine) TMB.
5. **Stop Solution**, 1 yellow cap vial, 6.0 ml of 1 mol/L sulfuric acid.
6. **Wash Solution Concentrate**, 1 transparent cap bottle, 25 ml (40X concentrated), PBS-Tween wash solution.
7. **IFU**, 1 copy.
8. **Plate Lid**: 1 piece.

**Materials required (but not provided)**

1. Microplate reader with 450nm and 620nm wavelength absorbent capability.
2. Microplate washer.
3. Incubator.
4. Plate shaker.
5. Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
6. Absorbent paper.
7. Distilled water

**Warnings and Precautions**

1. For in vitro diagnostic use only. For professional use only.
2. 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.
3. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
4. 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.
5. 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.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Do not use reagents beyond the labeled expiry date.
8. Do not mix or use components from kits with different batch codes.
9. If more than one plate is used, it is recommended to repeat the calibration curve.
10. It is important that the time of reaction in each well is held constant to achieve reproducible results.
11. Ensure that the bottom of the plate is clean and dry.
12. Ensure that no bubbles are present on the surface of the liquid before reading the plate.
13. The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

**Storage**

1. Store at 2-8°C.
2. Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
3. 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.
4. 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 labeled expiry date, whichever is earlier.

**Specimen collection and preparation**

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.
3. 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 heatin activated samples. Do not use samples and controls stabilized with azide.

4. Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
5. 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.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
8. 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.
9. 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.
10. Adjust the incubator to 37 °C.
11. Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
12. Don't use Substrate if it looks blue.
13. Don't use reagents that are contaminated or have bacteria growth.

**Quality control**

Each laboratory should 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**

1. Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
2. Add **25 µL of calibrators or samples** to each well.
3. Add **100 µL of enzyme conjugate** to each well.
4. Shake the microplate gently for 30 seconds to mix.
5. Cover the plate with a plate lid and incubate at 37 °C for 60 minutes.
6. Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add **350 µL of 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.
8. Add **100 µL of substrate** to each well.
9. Incubate at ambient temperature (18-25℃ in the dark for reaction for 20 minutes. Do not shake the plate after substrate addition.
10. Add **50 µL of stop solution** to each well.
11. Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
12. Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

**Calculation**

1. Record the absorbance obtained from the printout of the microplate reader.
2. Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
3. Plot the common logarithm of absorbance against concentration in ng/mL for each calibrator.
4. 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.

| Sample | Value (ng/mL) | Absorbance |
|---|---|---|
| Cal A | 0 | 0.015 |
| Cal B | 5 | 0.183 |
| Cal C | 10 | 0.492 |
| Cal D | 20 | 1.153 |
| Cal E | 50 | 2.107 |
| Cal F | 100 | 3.158 |
| Control1 | 5.84 | 0.23 |
| Control2 | 28.71 | 1.11 |
| Sample | 48.96 | 1.76 |

**Limitations - interference**

1. 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).
2. Criterion: Recovery within ± 15 % of initial value.
3. 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.
4. No interference was observed from rheumatoid factors up to a concentration of approx. 1100 IU/mL.
5. There is no high-dose hook effect at prolactin concentrations up to 220000 µIU/mL (10000 ng/mL).
6. In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
7. 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.
8. 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.
9. The release of prolactin is inhibited by dopamine, L-dopa and ergotamine derivatives.
10. 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.
11. 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.

12. 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.

**Expected values**

Men: 2.3 - 17.5 ng/mL

Women: 2.9 - 25.8 ng/mL

A study with the ATLAS 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 |

**Specific performance data**

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

**Precision**

Precision was determined using ATLAS 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 ATLAS Prolactin assay (y) with the Roche Cobas Prolatctin 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. 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 | Catalogue Number | | Temperature limit |
| IVD | In Vitro diagnostic medical device | | Caution |
| | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |