Prolactin
Enzyme immunoassay for the quantitative
determination of Prolactin in human serum or plasma
Only for in-vitro diagnostic use

Product Number: DNOV032 (96 Determinations)
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1. INTRODUCTION

Prolactin is a polypeptide hormone synthesised and secreted by the Adenohypophysis (anterior Pituitary gland) and the placenta. It is also produced in other tissues including the breast and the decidua. Pituitary prolactin secretion is regulated by neuroendocrine 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. INTENDED USE

Immunoenzymatic colorimetric method (ELISA) for quantitative determination of Prolactin in human serum or plasma.

3. PRINCIPLE OF THE ASSAY

The essential reagents required for an immunoenzymatic assay include high affinity and specificity antibodies (enzyme-labelled and immobilised) with different and distinct epitope recognition, in excess and native antigen.

Upon mixing monoclonal biotinylated antibody, the enzyme labelled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex.

The interaction is illustrated by the following equation:

$$\frac{K_a}{K_d} = \text{Rate constant of association}$$

$$\frac{K_d}{K_a} = \text{Rate constant of dissociation}$$

Simultaneously the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

This interaction is illustrated below:

$$\text{EnzAb}_{(p)} + \text{Ag}_{PRL} + BnAb_{(m)} \leftrightarrow \text{EnzAb}_{(p)}\text{Ag}_{PRL}BnAb_{(m)}$$

$$BnAb_{(m)} = \text{Biotinylated monoclonal antibody (excess quantity)}$$

$$\text{Ag}_{PRL} = \text{native Prolactin antigen (variable quantity)}$$

$$\text{EnzAb}_{(p)} = \text{Enzyme labelled polyclonal antibody (excess quantity)}$$

$$\text{EnzAb}_{(p)}\text{Ag}_{PRL}BnAb_{(m)} = \text{Antigen-antibody-sandwich complex}$$

$$K_a = \text{Rate constant of association}$$

$$K_d = \text{Rate constant of dissociation}$$

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By using several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4. MATERIALS

4.1. Reagents supplied

- **Microplate**: 12 breakapart 8-well snap-off strips coated with Streptavidin; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Anti-Prolactin-HRP conjugate**: 1 bottle containing 12 ml of horseradish peroxidase labelled polyclonal anti-Prolactin antibodies and biotinylated monoclonal anti-Prolactin antibodies.

- **TMB Substrate Solution**: 1 bottle containing 15 ml 3, 3’, 5, 5’-tetramethylbenzidine (H₂O₂-TMB 0.26 g/l) (avoid any skin contact).

- **Wash solution 50x conc**: one bottle containing 20 ml NaCl 45 g/l, Tween 20 55 g/l

- **Prolactin control**: 1 bottle containing 1 ml of a lot-specific control solution. The concentration is indicated on the label of the bottle.

- **Prolactin Standards**: 6 bottles, 1 ml each. The standards are calibrated against the (WHO 3rd IS 84/500) and have the following concentrations:
  - Standard 0: 0 ng/ml
  - Standard 1: 5 ng/ml
  - Standard 2: 10 ng/ml
  - Standard 3: 25 ng/ml
  - Standard 4: 50 ng/ml
  - Standard 5: 100 ng/ml

4.2. Materials supplied
- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE
The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark. After first use the standard solutions are still stable for another 6 months if stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

6.1. Microplate
The ready to use break apart snap-off strips are coated with streptavidine. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C.

6.2. Anti-Prolactin-HRP Conjugate
The bottle contains 12 ml of a solution with polyclonal anti-Prolactin antibodies conjugated with horse radish peroxidase and biotinylated monoclonal anti-Prolactin antibodies. It is ready to use.

6.3. Standards
Each of the 6 vials contains 1 ml standard solution of the concentration mentioned in 4.1. The standards are ready to use. After first use the standard solutions are still stable for another 6 months if stored at 2...8 °C.

6.4. TMB Substrate Solution
The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6.5. Stop Solution
The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26).

6.6. Wash Solution
Dilute contents of wash solution concentrate 50x to 1000 ml with distilled water in a suitable storage container. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days at 2…8°C.
6.7. Prolactin Control
The bottle contains 1 ml of a lot-specific control solution. The concentration is indicated on the label.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma samples with this assay. If the assay is performed within 48 hours after sample collection, the specimens should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. For samples with concentration above 100 ng/ml dilute 1/1 with Standard 0.

7.1. Precaution
- The reagents contain Proclin 300\textsuperscript{R} as a preservative.
- Do not use heavily haemolysed samples.
- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of Prolactin from 5 ng/ml to 100 ng/ml.

8. ASSAY PROCEDURE

8.1. Test Preparation
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well (e.g. A1) for the substrate blank
2 wells (e.g. B1+C1) for standard 0
2 wells (e.g. D1+E1) for standard 1
2 wells (eg. F1+G1) for standard 2
2 wells (eg. H1+A2) for standard 3
2 wells (eg. B2+C2) for standard 4
2 wells (eg. D2+E2) for standard 5
2 wells (e.g. F2+G2) for control

It is recommended to determine standards and patient samples in duplicate.
Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 50 µl standards, control and samples into their respective wells. Add 100 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour at room temperature (22...28°C).
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted Wash Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100 µl TMB Substrate Solution into all wells.
6. Incubate for exactly 15 min at room temperature in the dark.
7. Dispense 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 colour developed during the incubation turns into yellow.
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

8.2. Measurement
Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and patient sample in the distribution and identification plan.
9. RESULTS

9.1. Calculation of Results
Calculate the mean absorbance for each point of the standard curve and each sample.
Plot the values of absorbance of the standards against concentration.
Draw the best-fit curve through the plotted points (esp. Four Parameter Logistics or Sigmoide).
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentration expressed in ng/ml.

9.2. 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:

<table>
<thead>
<tr>
<th>Type</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.8 – 17.0 ng/ml</td>
</tr>
<tr>
<td>Female: menstrual cycle</td>
<td>1.2 – 19.5 ng/ml</td>
</tr>
<tr>
<td>Menopause</td>
<td>1.5 – 18.5 ng/ml</td>
</tr>
</tbody>
</table>

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

10. QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of Prolactin for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>σ</th>
<th>C.V. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>5.33</td>
<td>0.15</td>
<td>2.78</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>18.212</td>
<td>0.73</td>
<td>4.03</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>37.20</td>
<td>1.38</td>
<td>3.71</td>
</tr>
</tbody>
</table>

11.2. Specificity
The cross reaction of the antibody calculated at 50% according to Abraham:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>hProlactin</td>
<td>100.0 %</td>
</tr>
<tr>
<td>LH</td>
<td>none determined</td>
</tr>
<tr>
<td>FSH</td>
<td>none determined</td>
</tr>
<tr>
<td>hCG</td>
<td>none determined</td>
</tr>
<tr>
<td>TSH</td>
<td>none determined</td>
</tr>
<tr>
<td>hGH</td>
<td>none determined</td>
</tr>
</tbody>
</table>

11.3 Sensitivity
The lowest detectable concentration of Prolactin that can be distinguished from Standard 0 is 0.12 ng/ml at the 95% confidence limit.
11.4. Accuracy
The recovery of 3.13 – 6.25 – 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%.

11.5. Correlation
The NovaTec Prolactin ELISA (y) was compared to another commercially available Prolactin assay (x). Serum samples of 37 subjects were analysed. The linear regression curve was calculated:

\[ y = 1.01x + 1.94 \]
\[ r^2 = 0.957 \]

The new NovaTec Prolactin kit (y) was compared to the old version (x). 37 serum samples were analysed. The linear regression curve was calculated:

\[ y = 0.85x + 2.58 \]
\[ r^2 = 0.937 \]

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- 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.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- 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.

**WARNING:** In the used concentration Proclin 300\(^{\text{TM}}\) has hardly any toxicological risk upon contact with skin and mucous membranes!

**WARNING:** Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

12.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. LITERATURE
Cohen, K. L., Metabolism 26, 1165-1177 (1977)

14. ORDERING INFORMATION
Prod. No.: DNOV032 Prolactin Determination (96 Determinations)
SCHEME OF THE ASSAY
Prolactin

Test Preparation

- Prepare reagents and samples as described.
- Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
- Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th>Substrate blank</th>
<th>Standard 0 - 5</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0 - 5</td>
<td>-</td>
<td>50µl</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
</tr>
<tr>
<td>Conjugate</td>
<td>-</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

- Cover wells with foil supplied in the kit
- **Incubate for 1 h at room temperature (+22 - +28 °C)**
- Wash each well three times with 300 µl diluted wash solution

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>

- **Incubate for exactly 15 min at room temperature in the dark**

<table>
<thead>
<tr>
<th>Stop Solution</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>

- Shake the microplate gently
- Photometric measurement at 450 nm