Human Prolactin ELISA Kit

Enzyme Immunoassay for the quantification of human Prolactin in human plasma and milk samples

Catalog number: ARG81093

distributed in the US/Canada by:
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INTRODUCTION

Prolactin which is a secreted hormone is a growth regulator for many tissues, including cells of the immune system. It may also play a role in cell survival by suppressing apoptosis, and it is essential for lactation. Alternative splicing results in multiple transcript variants that encode the same protein. [provided by RefSeq, Aug 2011]

Prolactin acts primarily on the mammary gland by promoting lactation. [UniProt].

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of prolactin in human plasma and milk samples. The microtiter plate is coated with affinity purified prolactin antibody. Human Prolactin in samples or standards will react with antibody coated on the microtiter plate. After washing, a biotin-labeled polyclonal anti-Human Prolactin antibody is added and bound to the captured prolactin on the microtiter plate. Excess antibody is washed away and bound primary antibody is reacted with a Streptavidin conjugated to horseradish peroxidase (HRP). Following an additional washing step, TMB is added in the wells. HRP catalyzes TMB substrate solution, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of prolactin in test sample is directly proportional to the color intensity, which can be determined by extrapolation to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate (96 well)	4°C
Human Prolactin standard	1 vial (Lyophilized)	4°C
Anti-Human Prolactin primary antibody	1 vial (Lyophilized)	4°C
HRP-conjugated Streptavidin	1 vial (concentrated)	4°C
TMB substrate	10 ml	4°C (Protect from light)
10X Wash buffer	50 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 1N H_2SO_4 or 1N HCl
- TBS buffer: 0.1M Tris, 0.15M NaCl, pH 7.4
- Blocking buffer (BB): 3% BSA (w/v) in TBS
- Automated microplate washer (optional)
- Orbital microplate shaker

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Briefly spin down the Primary and HRP-conjugated Streptavidin before use.
- Store the kit at 4°C at all times.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X assay buffer into distilled water to yield 1X assay buffer. Mix well before use.
- Anti-Human Prolactin primary antibody: Add 10 ml of blocking buffer into primary antibody and vortex gently to completely dissolve contents.
- HRP-conjugated Streptavidin: Add 2.5 µl of concentrated Streptavidin in

2.5 ml of blocking buffer to generate a 1:1,000 dilution. Add 0.4 ml of 1:1,000 dilution to 9.6 ml of blocking buffer to generate a 1:25,000 dilution.

- **Standard:** Reconstitute standard by adding 1 ml blocking buffer (BB) to vial and mix gently to completely dissolve contents. This will result in 500 ng/ml stock solution. Dilute standard as according to the table below:

Prolactin concentration (ng/ml)	Dilutions
100	800 µl (BB) +200 µl (From stock vial)
50	500 µl (BB) + 500 µl (100 ng/ml)
25	500 µl (BB) + 500 µl (50 ng/ml)
10	600 µl (BB) + 400 µl (25 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2.5	500 µl (BB) + 500 µl (5 ng/ml)
1	600 µl (BB) + 400 µl (2.5 ng/ml)
0.5	500 µl (BB) + 500 µl (1 ng/ml)
0.25	500 µl (BB) + 500 µl (0.5 ng/ml)
0	500 µl (BB) (Zero point to determine background)

Note: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

- **Sample:** If the measuring absorbance of samples is higher than the highest standard or if the unknown is thought to have high prolactin levels, dilute the samples with blocking buffer before assay and assay again. Normal plasma should not require dilution before use in this assay. A 1:2 to 1:4 dilution for breast milk is suggested to ensure that resulting values fall within the linear range of the assay.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards or unknown samples into appropriate wells. Shake the plate on a microplate shaker at 300 rpm for 30 minutes at RT.
3. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× assay buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl of primary antibody into each well. Shake the plate on a microplate shaker at 300 rpm for 30 minutes at RT.
5. Wash as according to step 3.
6. Add 100 µl of HRP-conjugated Streptavidin (1:25,000 diluted) into each well. Shake the plate on a microplate shaker at 300 rpm for 30 minutes at RT.
7. Wash as according to step 3.
8. Add 100 µl of TMB Reagent to each well. Shake plate at 300 rpm for 5-20 minutes at RT in dark. Substrate will change from colorless to different strengths of blue.
9. Add 50 µl of 1N H_2SO_4 or HCl to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking the plate.

10. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

6. Expected Values: The concentration of prolactin in normal human plasma varies between the sexes and is considerably higher during pregnancy. Normal values as defined by the NIH are as follows:

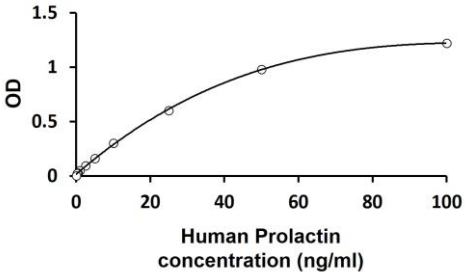
- Males: 2-18 ng/mL
- Nonpregnant females: 2-29 ng/mL
- Pregnant women: 10-209 ng/mL

The concentration of prolactin in human breast milk is relatively high in the days immediately after childbirth, peaking at 157 ± 18 ng/mL on the third day

following delivery. Prolactin concentrations then fall quickly to 24 ng/mL by the 13 th postpartum day.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

Assay range: 0-100 ng/ml

Minimum Detectable Concentration: 0.25 ng/ml

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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