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Human Prolactin Antigen Assay
Catalog # (HPRLKT)
Strip well format. Reagents for up to 96 tests.
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INTENDED USE
This human prolactin antigen assay is intended for the quantitative determination of prolactin antigen in human plasma or breast milk.
 For research use only.

BACKGROUND
Prolactin (PRL) is a 199 aa, 23kD peptide hormone that is secreted primarily by the pituitary gland in both males and females, though its major roles are in pregnancy and lactation [1,2]. Prolactin may have a role in breast cancer development, with higher prolactin levels correlating with postmenopausal breast cancer risk [3].

ASSAY PRINCIPLE
Human prolactin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotinlyated anti-human prolactin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total prolactin present in the samples, is reacted with peroxidase-conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human prolactin. Color development is proportional to the concentration of prolactin in the samples.

REAGENTS PROVIDED
- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human prolactin antibody, blocked and dried.
- **10X Wash Buffer:** 1 bottle of 50ml
- **Human Prolactin standard:** 1 vial lyophilized standard
- **Anti-human Prolactin primary antibody:** 1 vial lyophilized biotinylated antibody
- ♦ **Peroxidase conjugated Streptavidin**
  1 vial of concentrated Streptavidin-HRP
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY
Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED
- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS
- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS
- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION
Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE
Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard
Reconstitute standard by adding 1 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 500ng/ml standard solution.

Dilution table for preparation of human prolactin standard:

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

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition
Remove microtiter plate from bag and add 100µl Prolactin standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total human prolactin in the 0.25-100 ng/mL range. Samples with human prolactin levels above 100ng/mL should be diluted in blocking buffer before use. 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.

Primary Antibody Addition
Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition
Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of 1:1,000 dilution to 9.6ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation
Add 100µl TMB substrate to all wells and shake plate for 5-20 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

Measurement
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results
Plot A_{450} against the amount of Prolactin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of Prolactin in the unknowns can be determined from this

curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor. A typical standard curve (EXAMPLE ONLY):

Human Prolactin
0-100ng/ml

Human Prolactin
0-10ng/ml
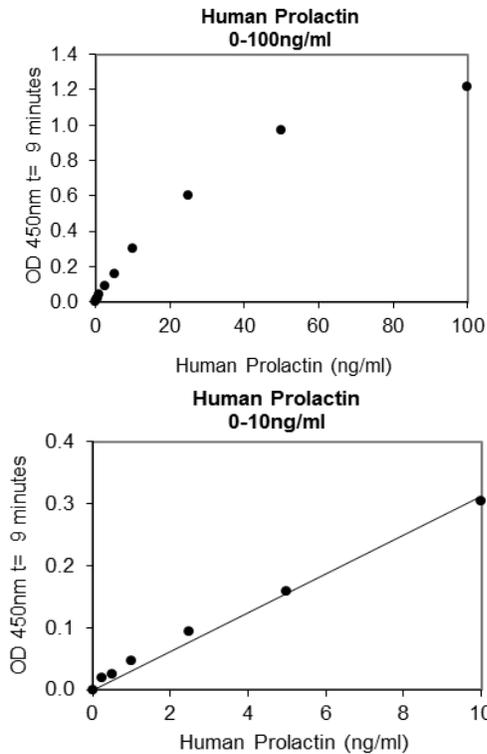
EXPECTED VALUES

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 13th postpartum day [4].

PERFORMANCE CHARACTERISTICS

Sensitivity: These studies are currently in progress. Please contact us for more information.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Goffin V, *et al*.: Annu Rev Physiol. 2002, 64:47-67.
2. Shiu RP, *et al*. Annu Rev Physiol. 1980, 42-83-96.
3. Hankinson SE, *et al*. J Natl Cancer Inst. 1999, 91:629-634.
4. Healy DL, *et al*. Am J Physiol, 1980, 238(1):E83-86.

Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.25ng/mL	0.5ng/mL	1ng/mL	2.5ng/mL	5ng/mL	10ng/mL	25ng/mL	50ng/mL	100ng/mL		
B	0	0.25ng/mL	0.5ng/mL	1ng/mL	2.5ng/mL	5ng/mL	10ng/mL	25ng/mL	50ng/mL	100ng/mL		
C												
D												
E												
F												
G												
H												

SAMPLE INSERT
Refer to kit box for
lot specific instructions