

# **LSBio™ Human PRL / Prolactin ELISA Kit**

**Catalog No. LS-F10428**

## **User Manual**

**Please Read the Manual Carefully  
Before Starting your Experiment**



For research use only. Not approved for use in humans or for clinical diagnosis.

## 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, biotinylated 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<sub>2</sub>SO<sub>4</sub> 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  $\leq -20^{\circ}\text{C}$ . 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 200ng/ml standard solution.

Dilution table for preparation of human prolactin standard:

Prolactin concentration (ng/ml)	Dilutions
100	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (200ng/ml)
50	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (100ng/ml)
25	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (50ng/ml)
10	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (25ng/ml)
5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (10ng/ml)
2.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (5ng/ml)
1	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (2.5ng/ml)
0.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (1ng/ml)
0.25	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (0.5ng/ml)
0	500 $\mu\text{l}$ (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 100 $\mu\text{l}$  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 300 $\mu\text{l}$  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 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5 $\mu\text{l}$  of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.8ml of 1:1,000 dilution to 9.2ml of blocking buffer to generate a 1:12,500 dilution. Add 100 $\mu\text{l}$  of the 1:12,500 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Substrate Incubation

Add 100 $\mu\text{l}$  TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 $\mu\text{l}$  of 1N  $\text{H}_2\text{SO}_4$  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

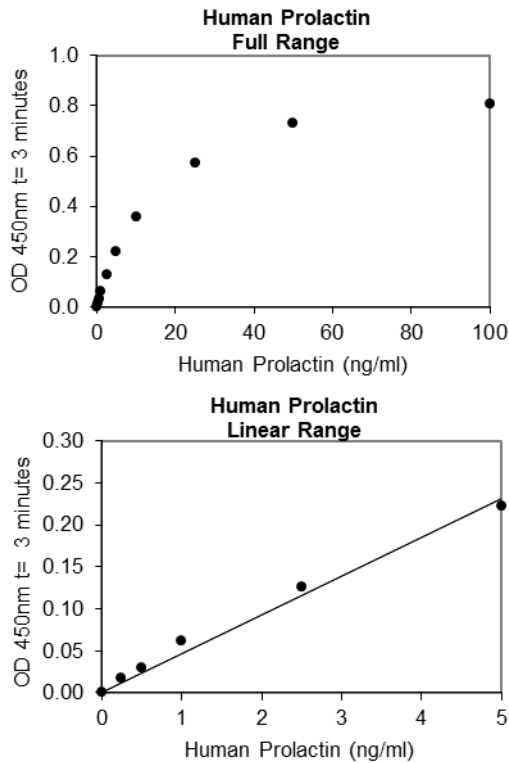
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ( $A_{450}$ ).

### 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):



## 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  $157 \pm 18$  ng/mL on the third day following

delivery. Prolactin concentrations then fall quickly to 24 ng/mL by the 13<sup>th</sup> postpartum day [4].

## PERFORMANCE CHARACTERISTICS

**Sensitivity:** The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD<sub>450</sub>: 0.072-0.083) and calculating the corresponding concentration. The MDD was 0.138 ng/ml.

**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**

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





**Important Note:** During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

**Warning:** This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

**Returns, Refunds, Cancellations:** Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan BioSciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancellations may be subject to a 30% restocking fee.

**Conditions & Warranty:** All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or guarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages. LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

**For research use only. Not approved for use in humans or for clinical diagnosis.**



2401 Fourth Avenue Suite 900 Seattle, WA 98121

Tel: 206.374.1102

Fax: 206.577.4565

[Technical.Support@LSBio.com](mailto:Technical.Support@LSBio.com)