Prolactin (human) ELISA Kit

Item No. 500730

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
400730	Prolactin Standard 1 (0 ng/ml)	1 vial/1 ml
400731	Prolactin Standard 2 (5 ng/ml)	1 vial/1 ml
400732	Prolactin Standard 3 (10 ng/ml)	1 vial/1 ml
400733	Prolactin Standard 4 (25 ng/ml)	1 vial/1 ml
400734	Prolactin Standard 5 (50 ng/ml)	1 vial/1 ml
400735	Prolactin Standard 6 (100 ng/ml)	1 vial/1 ml
400736	Streptavidin Precoated Plate	1 plate
400737	Anti-Prolactin-HRP + Anti-Prolactin-Biotin Conjugate	1 vial/12 ml
400738	TMB Substrate Solution	1 vial/15 ml
400739	Stop Solution	1 vial/15 ml
400835	Wash Solution (50X)	1 vial/50 ml
400836	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Prolactin (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
Email:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. Materials used for **Sample Preparation** (see page 11).

INTRODUCTION

Background

Prolactin is a polypeptide hormone synthesized and secreted by the anterior pituitary gland, placenta, decidua, and by various immune system cells, such as T cells, B cells, and NK cells. It is present in several body fluids, including plasma, amniotic fluid, milk, mucosal secretions, and cerebrospinal fluid. Prolactin has many functions, the most important of which is to stimulate the mammary glands to produce milk (lactation). Other functions may include the stimulation of surfactant in the fetal lungs at the end of pregnancy, induction of immune tolerance of the fetus during pregnancy, as well as other immuno-regulatory functions, regulation of reproductive functions, and may also have a role in breast cancer development. During pregnancy, increased estrogen promotes the production of prolactin, which in turn promotes the maturation of the mammary glands. High levels of prolactin also tend to suppress the ovulatory cycle by inhibiting the secretion of FSH and GnRH, and can have inhibitory effects on gonadal function leading to hypogonadism, and sometimes causing erectile dysfunction in men.

About This Assay

Cayman's Prolactin (human) ELISA Kit is an immunometric (*i.e.*, sandwich) ELISA that can be used to measure prolactin within the range of 0.12-100 ng/ml. This assay offers specific and sensitive analysis of prolactin in human serum and plasma, and has not been validated for other types of samples.

Principle of the Assay

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with streptavidin. Samples and/or standards, biotinylated capture antibody and an HRP-labeled detection antibody (anti-prolactin-HRP) are incubated in the wells. The biotinylated-capture antibody will bind both the streptavidin on the plate and any prolactin introduced into the well, whereas the detection antibody will bind a different epitope on the prolactin molecule. The entire complex is immobilized onto the wells by the streptavidin-biotinylated antibody interaction. After washing away excess, unbound reagents, the concentration of prolactin is determined by measuring the enzymatic activity of HRP by adding the substrate tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color is directly proportional to the amount of bound anti-prolactin-HRP, which in turn is proportional to the amount of prolactin.

$$\text{Absorbance} \propto [\text{Anti-Prolactin HRP}] \propto [\text{Prolactin}]$$

A schematic of this process is shown in Figure 1, on page 8.

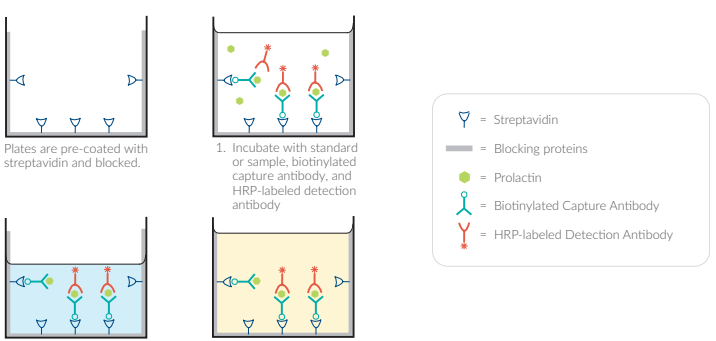


Figure 1. Schematic of the ELISA

Definition of Key Terms

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C.

Wash Buffer Preparation

50 ml vial Wash Solution (50X; Item No. 400835): Dilute to a total volume of 2,500 ml with distilled or deionized water.

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:50.

Sample Preparation

Human serum and plasma can be used directly in the assay.

NOTE: Do not use heavily hemolyzed sample. Store samples refrigerated (2°C-8°C) for a maximum of 48 hours. If samples cannot be assayed within this time, store them at -20°C to -70°C. Avoid repetitive freeze-thaw cycles. Mix thawed samples well before testing.

When assayed in dulpicate, 50 µl is required. If the concentration of prolactin in the sample is greater than 100 ng/ml, dilute an aliquot of the sample with Standard 1 (0 ng/ml).

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

NOTE: It is very important to bring all reagents, samples, and standards to room temperature (22-28°C) before starting the assay.

Prolactin Standard (Item No. 400730-400735)

Each of the six vials contains 1 ml standard solution at concentrations listed in the **Materials Supplied** section (see page 3), as well on each vial. The standards are ready to use. After opening, the standard solutions are stable for six months if stored at 4°C.

Anti-Prolactin-HRP + Anti-Prolactin-Biotin Conjugate (Item No. 400737)

This vial contains 12 ml of a ready-to-use mixture of HRP-labelled Anti-Prolactin and biotin-labelled Anti-Prolactin antibodies.

TMB Substrate Solution (Item No. 400738)

This vial contains 15 ml of a ready-to-use tetramethylbenzidine/hydrogen peroxide substrate solution. When stored in the dark at 4°C, the solution is stable for up to six months after opening. *The solution should be colorless or have a slight blue tinge. If it is blue, it may have become contaminated and should not be used.*

Stop Solution (Item No. 400739)

This vial contains 15 ml 0.15 M sulfuric acid and is ready to use.

Plate Set Up

The 96-well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, plate the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and a six point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 2, see below. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest you record the contents of each well on the template sheet provided (see page 22).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	2	2	10	10	18	18	26	26	34	34
B	S2	S2	3	3	11	11	19	19	27	27	35	35
C	S3	S3	4	4	12	12	20	20	28	28	36	36
D	S4	S4	5	5	13	13	21	21	29	29	37	37
E	S5	S5	6	6	14	14	22	22	30	30	38	38
F	S6	S6	7	7	15	15	23	23	31	31	39	39
G	Blk	Blk	8	8	16	16	24	24	32	32	40	40
H	1	1	9	9	17	17	25	25	33	33	41	41

Blk - Blank
S1-S6 - Standards 1-6
1-41 - Samples

Figure 2. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

NOTE: Perform all assay steps in the order given and without appreciable delays between steps.Pipetting samples should not extend beyond ten minutes to avoid assay drift. TMB Substrate Solution and Stop Solution should be added in the same sequence.

Addition of the Reagents

1. **Prolactin Standards**

   Add 50 µl of each standard to appropriate wells.

2. **Samples**

   Add 50 µl of sample to appropriate wells.

3. **Anti-Prolactin Conjugate**

   Add 100 µl conjugate to each well, except the Blk wells.

Incubation of the Plate

Cover the plate with the plastic film and incubate at room temperature (22°C-28°C) for one hour.

Development of the Plate

1. Empty the wells and wash three times with 300 µl of diluted Wash Buffer. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of TMB Substrate Solution to each well of the plate, including the Blk wells.
3. **Incubate for exactly 15 minutes** at room temperature in the dark.
4. DO NOT WASH THE PLATE OR EMPTY THE WELLS. Add 100 µl Stop Solution to all wells and in the same order and same rate as the addition of TMB Substrate in Step 2.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Calculations

Standard Curve & Determination of Sample Concentration

Average the absorbance values of the Blk wells and subtract this value from the absorbance readings of each standard and sample well.

Using computer data reduction software, plot O.D. *versus* concentration for standards (S1-S6) and fit the data with a 4-parameter logistic equation, or alternatively, a sigmoid equation. Interpolate the concentration of your samples from the standard curve and be sure to correct for any dilution of the sample prior to addition to the well of the plate.

Reference Values

The following are expected ranges of prolactin in human serum or plasma:

Males:	1.8-17.0 ng/ml
Females:	
Pre-Menopause	1.2-19.5 ng/ml
Post-Menopause	1.5-18.5 ng/ml

NOTE: It is possible that some females in the sample population were using oral contraceptives, which may affect the results.

Performance Characteristics

Sensitivity

The minimal detectable concentration of prolactin by this assay is estimated to be 0.12 ng/ml.

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

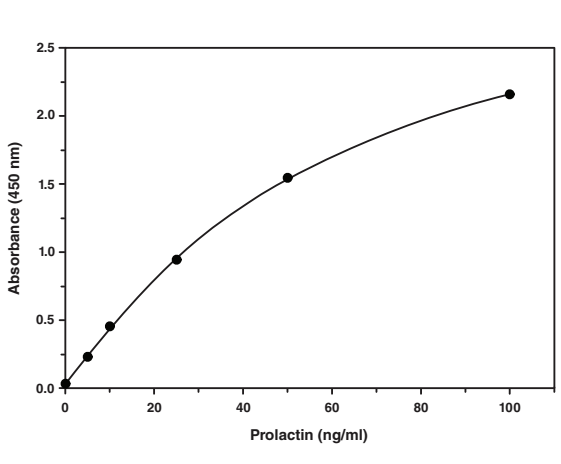


Figure 3. Typical standard curve

Precision

1. **Intra-assay variation**

   The precision within an assay was determined by 20 replicate determinations of three different control sera in the same assay.

Serum Sample	1	2	3
Number of Replicates	20	20	20
Mean Prolactin (ng/ml)	5.33	18.22	37.2
Standard Deviation	0.15	0.73	1.38
Coefficient of Variation (%)	2.8	4.03	3.71

Table 1. Intra-Assay Variation

2. **Inter-assay variation**

   The precision between assays was determined by replicate measurements of three different control sera in different assays.

Serum Sample	1	2	3
Number of Replicates	10	10	10
Mean Prolactin (ng/ml)	5.46	17.72	36.29
Standard Deviation	0.3	0.91	1.67
Coefficient of Variation (%)	5.49	5.16	4.6

Table 2. Inter-Assay Variation

Cross Reactivity:

Substance	Cross Reactivity
Prolactin	100%
Luteinizing Hormone	None detected
FSH	None detected
hCG	None detected
TSH	None detected
hGH	None detected

Table 3. Cross Reactivity of the Prolactin Assay

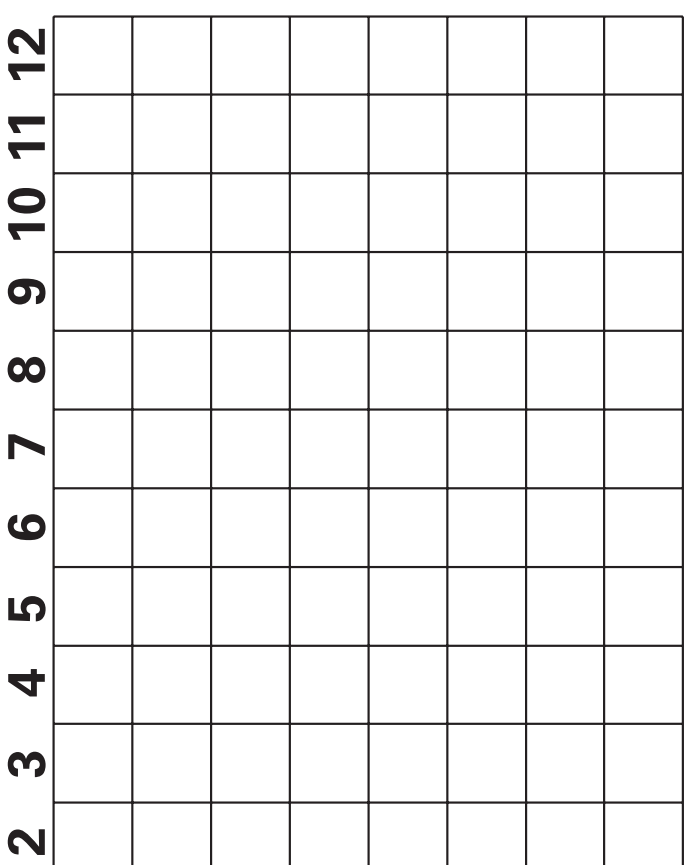
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal	A. Omission of key reagent B. Washes too stringent C. Incubation times inadequate D. Plate reader settings not optimal E. Incorrect assay temperature	A. Check that all reagents have been added in the correct order B. Use an automated plate washer if possible C. Use recommended incubation times D. Verify the wavelength and/or filter settings in the plate reader E. Use recommended incubation temperature; bring substrates to room temperature before use
High background	Inadequate washing	Ensure all wells are filled with Wash Buffer and are aspirated completely
Poor standard curve	A. Wells not completely aspirated B. Reagents poorly mixed C. Technique problem	A. Completely aspirate wells between steps B. Be sure that reagents are thoroughly mixed C. Proper mixing of reagents and wash steps are critical

References

1. Goffin, V., Binart, N., Touraine, P., *et al.* Prolactin: The new biology of an old hormone. *Annu. Rev. Physiol.* **64**, 47-67 (2002).
2. Batzer, F.R. Hormonal evaluation of early pregnancy. *Fert. Steril.* **34(1)**, 1-13 (1980).
3. Cohen, K.L. Metabolic, endocrine, and drug-induced interference with pituitary function tests: A review. *Metabolism* **26(10)**, 1165-1177 (1977).
4. Wisdom, G.B. Enzyme-immunoassay. *Clin. Chem.* **22(8)**, 1243-1255 (1976).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.