

ELISA kits available from ADI (see details at the web site)

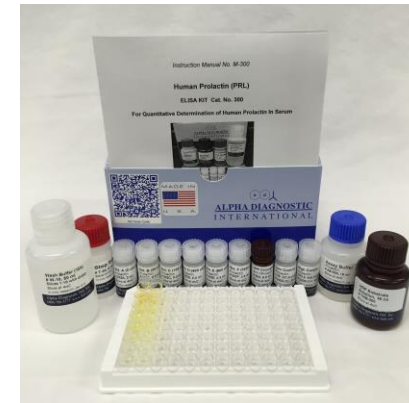
#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Folicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (ft4)
#1650	Human free triiodothyronine (ft3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnlone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-300

Human Prolactin (PRL)

ELISA KIT Cat. No. 300, 96 Tests

For Quantitative Determination of Human Prolactin In Serum



For In Vitro Research Use Only



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Human Prolactin (PRL) ELISA KIT Cat. No. 300

Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	
Anti-hProlactin coated strip plate (96 wells) , #301	1 plate
Prolactin Std. A (0 µIU/ml), 2 ml, #302A	1 vial
Prolactin Std. B (20 µIU/ml), 0.3 ml, #302B	1 vial
Prolactin Std. C (100 µIU/ml) 0.3 ml, #302C	1 vial
Prolactin Std. D (400 µIU/ml) 0.3 ml, #302D	1 vial
Prolactin Std. E (800 µIU/ml) 0.3 ml, #302E	1 vial
Prolactin Std. F (3200 µIU/ml) 0.3 ml, #302F	1 vial
Prolactin Controls Low & High (exact values printed on vials) #0300CL-CH, 0.3 ml/vial, store at 2-4oC. Standards and controls are calibrated to WO 3 rd IS 80/500 (21.5 µIU=1 ng)	
Anti-hProlactin-HRP Conjugate(50X) 0.3 ml , #303	1 vial
Assay Buffer , 15 ml, #AB-300	1 bottle
Wash buffer (10X) , 50 ml (dilute 1:10 with water), #W-10	1 bottle
HRP Substrate Solution , 16 ml; #TMB-300	1 bottle
Stop solution, 6 ml , #T-30	1 bottle
Complete Instruction Manual, M-300	1

Introduction

Prolactin (PRL) is a polypeptide hormone synthesized by the lactotropic cells of the anterior pituitary gland. Structurally, it is similar to two other polypeptide hormones namely, growth hormone and placental lactogen. PRL is a polypeptide hormone containing 199 amino acids, while growth hormone and placental lactogen each have 191 amino acids. There is about 100 µg of prolactin in the human pituitary gland, which is very small amount when compared to Growth hormone, which is present in 8-10 mg. Prolactin target organ is the breast (mammary gland). Its main physiological action is not only to initiate but also to sustain lactation. The hypothalamus secretes dopamine, which has a direct effect of inhibition on the secretion of PRL. If dopamine is not available or absent the secretion of PRL is autonomous. PRL increases during sleep and pregnancy. In women giving birth and they do not breast feed then PRL decreases to its normal level in a short time. During breast feed, PRL remains elevated for about 3 months. Also during pregnancy the size of the pituitary gland increases which is due to the proliferation of lactotropic cells.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum concentration of prolactin detected using this assay is **10 µIU/ml**. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Sample	Mean µIU/ml	SD	CV%
1	202	14	6.9
2	586	68	11.6
3	1320	136	10.3

Inter-assay precision:

Sample	Mean µIU/ml	SD	CV%
1	237	18	7.6
2	589	85	14.4
	1725	277	13.2

3. RECOVERY

A known amount of prolactin was added to three patient sera (with original prolactin concentrations) and the total prolactin concentrations measured. The assay showed good mean recoveries 81-125%

4. LINEARITY

A patient sample (with original prolactin concentrations) was diluted (1:2, 1:4, 1:8, and 1:16) with the zero standard and their prolactin values determined. The samples showed excellent mean recoveries respectively of 85-104%.

5. SPECIFICITY

The specificity of prolactin ELISA kit was determined by measuring interference from high concentrations of hGH (up to 1000 µg/ml), HCG (up to 2500 mIU/ml), hLH (up to 2500 mIU/l), hTSH (up to 2500 mIU/ml), and hFSH (up to 4000 mIU/ml). These hormones had minimal (0-2.5%) interference in the prolactin assay.

6. ANIMAL SPECIFICITY

Human prolactin ELISA kit has not been tested in other species (rat, mouse, monkey etc). It may work in other species provide the antibodies used in the kit are reactive with a given species.

General Reference: Aubert ML et al (1974) Acta Endocrinol. 77, 460; Cowden EA et al (1979) Annals Clin. Chem. 16, 1113; Frantz AG et al (1978) New Engl J. Med. 298, 201; Friesen H et al (1973) Ann. Rev. med. 24, 251; Jacobs LP et al (1974) J Clin. Endocrinol. 33, 996; Shome B et al (1977) J Clin. Endocrinol. Metabol. 45, 1112; Tyson JE et al (1972) Am. J. Obstet. Gynecol. 113, 14.

ADI's Human Prolactin ELISA kit publications

Krikun G 2004 Endocrinology, 145: 2291 – 2296

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Once opened/used standards are stable for two months at 2-8°C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

1. **TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE). Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml water). Dilute HRP conjugate stock 1:50 in assay buffer (40 ul of conjugate conc. In 2 ml of assay buffer)**

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.
3. Pipet **100 µl of Diluted Ab-enzyme** conjugate into each well.. Mix gently for 5-10 seconds. Cover the plate and **incubate on a plate shaker** (approx. 200 rpm) for **60 minutes** at room temperature (25-28°C).
4. Aspirate and **wash the wells 3 times** with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate washer for better consistency and to prevent contamination. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing. Appropriate precautions must be observed to prevent contamination.
5. Dispense **150 ul TMB substrate per well**. Mix gently for 5 seconds, cover the plate and incubate on a plate shaker for **10-15 minutes** at room temperature. Blue color develops in standards/controls and in positive wells.
6. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals (color turns yellow). Mix gently for 5-10 seconds.
7. Measure the **absorbance at 450 nm** using an ELISA reader. Color is stable for at least 30 min after stopping.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples containing more than 3200 µIU/ prolactin must be diluted with the zero standard (standard A) and the results obtained should be multiplied by the appropriate dilution factor.

EXPECTED VALUES

Normal range: The normal range for plasma prolactin is 67-360 µIU/ml for men and 55-2500 µIU/ml for women. There is apparently an estrogen related rise at puberty and a corresponding fall at menopause. Prolactin level is increased progressively at throughout the pregnancy to 10-20 times the non-pregnant values. The prolactin value falls after delivery, declining to non-pregnant level by 3-4 weeks post-partum. The decline is more gradual in suckling mothers taking several months to fall to adult levels.

Hypolactinemia in Sheehan's syndrome and in others types of hypo-pituitaries. Base-line prolactin measurements may not reliably differentiate from low normal values, but serum prolactin will not rise often after TRH stimulation in affected patients.

Hyperlactinemia is a much more common problem than prolactin deficiency. The principle direct symptom of prolactin excess is galactorrhea or nonpuerperal lactation, but the correlation between elevates levels and lactation is poor.

Overproduction of prolactin is the most common hormonal abnormality of pituitary neoplasm. Whether or not galactorrhea is found, serum prolactin should be measured in every patient with enlarged sella or with secondary amenorrhea. Values about 300 ng/ml regularly indicate a pituitary tumor, whether or not the sella is enlarged. In patients with values 40-200 ng/ml, polytomography of the sella may reveal small irregularities that suggest a pituitary microadenoma. Levels below 40 ng/ml are more enigmatic, although such minimal elevations have been associated with pituitary tumor.

Evaluation of amenorrhea, infertility or impotence. Secondary features of hyperlactinemia may cause a short luteal phase, anovulation, oligomenorrhea, and amenorrhea in the female and impotence in the male. These consequences of prolactin excess are probably caused by inhibition of gonadotropin secretion.

Hyperlactinemia often occurs secondary to the use of phenothiazines, monoamine oxidase inhibitors or other drugs that influence hypothalamic or adrenergic function. Hyperlactinemia can occur either during or shortly after discontinuing use of oral contraceptives.

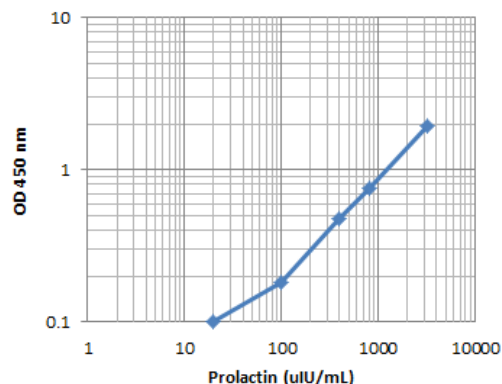
Primary hypothyroidism can cause hype-prolactinemia, but prolactin values seldom exceed 50 ng/ml.

Renal failure is also associated with prolactin elevation.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (μIU/mL)	Av. Abs.	Calculated Conc'n
A1, A2	Std. A, 0	0.080	
B1, B2	Std. B, 20	0.101	
C1, C2	Std. C, 100	0.182	
D1, D2	Std. D, 400	0.479	
E1, E2	Std. E, 800	0.760	
F1, F2	Std. F, 3200	1.937	

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate prolactin concentrations. Read off the prolactin concentrations of the control and patient samples. If ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve.

1 ng of human PRL equals 21.5 μIU.

After the pregnancy, the gland decreases to its normal size.

In patients where tumors secrete prolactin there is a remarkable increase in the PRL level, which then decreases the secretion of gonadotropin resulting in infertility. If the pituitary gland is deficient it leads to failure of lactation. In Sheehan's syndrome the pituitary gland is deficient, therefore, the PRL level is reduced. A few conditions where an increase in prolactin level is found include: Hyprelactinemia, adenomas of pituitary gland; sleep; pregnancy; hypothyroidism, prolactinomas and stress. Prolactinomas are pituitary secreting prolactin found most frequently in females and lead to amenorrhea which could be primary or secondary and given rise to a decrease in gonadotropin secretion by the pituitary. In me some degree of impotency accompanied by a low testosterone level occurs, followed by azospermia.

PRINCIPLE OF THE TEST

Prolactin ELISA kit is based on sequential binding of human prolactin from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate (TMB) is added and color (blue) developed. The enzymatic reaction (blue color) is directly proportional to the amount of prolactin present in the sample. The reaction is terminated by adding stop solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 μl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Prolactin ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions. The Controls and Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Reagent Preparation

Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml. Store at 4°C.

Prepare 1X solution HRP conjugate. Dilute 20 ul stock conjugate per ml of assay buffer. (200 ul in 10 ml for complete 96-well plate). Prepare in required volumes and do not store working conjugate beyond the assay date.