LDN Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

LDN®

Instructions for use

Prolactin ELISA

REF FR E-2900

Σ 96

+2 +8 °C

IVD

CE

Prolactin ELISA

INTENDED USE

For the quantitative determination of prolactin (PRL) in human serum by an enzyme immunoassay. For *in vitro* use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for prolactin is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of prolactin is conjugated to horse radish peroxidase (HRP). Prolactin from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of prolactin in the sample. A set of standards is used to plot a standard curve from which the amount of prolactin in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

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 containing 199 amino acids, while growth hormone and placental lactogen each have 191 amino acids. There is approximately 100 µg of prolactin in the human pituitary gland, which is a very small amount when compared to growth hormone, which is present at 8-10 mg.

The target organ of prolactin 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 of the secretion of PRL.

If dopamine is not available or absent the secretion of PRL is autonomous. Clinical trends:

- In patients where tumours 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 increases in prolactin levels are found include: hyperprolactinemia, adenomas of the pituitary gland, sleep, pregnancy, hypothyroidism, prolactinomas and stress.
- Prolactinomas are pituitary tumours secreting prolactin, found most frequently in females. In females they lead to amenorrhoea, which could be primary or secondary and give rise to a decrease in gonadotropin secretion by the pituitary. In men some degree of impotency accompanied by a low testosterone level occurs, followed by azospermia.

PROCEDURAL CAUTIONS AND WARNINGS

- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- A calibrator curve must be established for every run.
- The controls should be included in every run and fall within established confidence limits.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
- When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

- All the reagents within the kit are calibrated for the determination of prolactin in human serum. The kit is not calibrated for the determination of prolactin in saliva, plasma or other specimens of human or animal origin.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
- Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.
- The measurement of prolactin may also include the measurement of its other structural forms (big prolactin, macroprolactin, etc.). As a result, patients exhibiting elevated prolactin levels may require further investigations to make a proper diagnosis.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4^{o}C for up to 24 hours or at -10^{o}C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes to dispense 25, 50, 100 and 300 µL
- Disposable pipette tips
- Distilled or deionized water
- Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 11).

REAGENTS PROVIDED

AA E-0030 WASH-CONC 10x **Wash Buffer Concentrate – X10**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume:	50 mL/bottle
Storage:	Refrigerate at 2-8^{o}C
Stability:	12 months or as indicated on label.
Preparation:	Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

AA E-0055 SUBSTRATE **TMB Substrate** - Ready To Use.

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	16 mL/bottle
Storage:	Refrigerate at 2-8^{o}C
Stability:	12 months or as indicated on label.

AA E-0080 STOP-SOLN **Stopping Solution** - Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 mL/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Calibrators and **Controls**- Ready To Use.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. no.	Symbol	Calibrator	Concentration	Concentration	Volume/Vial
FR E-2901	STANDARD A	**Calibrator A**	0 µIU/mL	0 ng/mL	2.0 mL
FR E-2902	STANDARD B	**Calibrator B**	20 µIU/mL	0.77 ng/mL	0.3 mL
FR E-2903	STANDARD C	**Calibrator C**	100 µIU/mL	3.84 ng/mL	0.3 mL
FR E-2904	STANDARD D	**Calibrator D**	400 µIU/mL	15.38 ng/mL	0.3 mL
FR E-2905	STANDARD E	**Calibrator E**	800 µIU/mL	30.75 ng/mL	0.3 mL
FR E-2906	STANDARD F	**Calibrator F**	3200 µIU/mL	123 ng/mL	0.3 mL
FR E-2951	CONTROL 1	**Control 1**	Refer to vial labels for expected value and acceptable range!		0.3 mL
FR E-2952	CONTROL 2	**Control 2**			0.3 mL

Contents: Prolactin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of prolactin. Calibrated against World Health Organization (WHO) 3rd IS 80/500.

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

FR E-2913 ASSAY-BUFF **Assay Buffer** - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 15 mL/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

FR E-2931 96 **Mouse Anti-Prolactin Antibody Coated Microwell Plate-Break Apart Wells** - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

FR E-2940 CONJUGATE-CONC 50x **Mouse Anti-Prolactin Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate – X50**

Contents: Anti-PRL monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.3 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	Prepare **working solutions** of the **anti Prolactin-HRP conjugate** and **wash buffer**.
2.	Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3.	**Pipette 25 µl** of each **calibrator, control and specimen sample** into correspondingly labelled wells in duplicate.
4.	Pipette **100 µL** of the **conjugate working solution** into each well. *(We recommend using a multichannel pipette).*
5.	**Incubate** on a plate shaker (approximately 200 rpm) for **1 hour** at **room temperature**.
6.	Wash the wells **3 times** with prepared wash buffer (**300 µL**/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry *(The use of a washer is recommended).*
7.	Pipette **150 µL** of **TMB substrate** into each well at timed intervals.
8.	Incubate the plate on a plate shaker at **room temperature** for **10-15** minutes. *(or until Calibrator A attains dark blue colour for desired OD).*
9.	Pipette **50 µl** of **stopping solution** into each well at the same timed intervals as in step 7.
10.	Read the plate on a microwell plate reader at **450 nm** within 20 minutes after addition of the stopping solution.
⚠	*If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.*

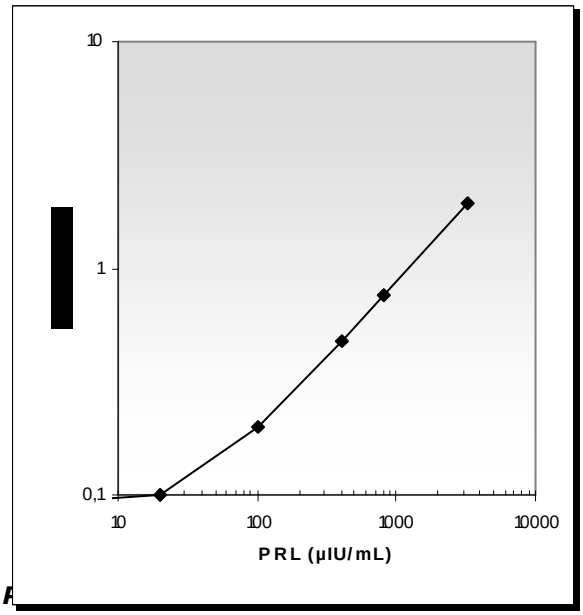
CALCULATIONS

- Calculate the mean optical density of each calibrator duplicate.
- Calculate the mean optical density of each unknown duplicate.
- Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, controls and serum samples.
- Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
- Read the values of the unknowns directly off the calibrator curve.
- If a sample reads more than 3200 µIU/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (µIU/mL)
A	0.081	0.079	0.080	0
B	0.099	0.102	0.101	20
C	0.181	0.182	0.182	100
D	0.477	0.481	0.479	400
E	0.769	0.751	0.760	800
F	1.858	2.016	1.937	3200
Unknown	0.442	0.469	0.456	383.458

TYPICAL CALIBRATOR CURVE
Sample curve only. **Do not** use to calculate results.



SENSITIVITY
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the dbc prolactin ELISA kit is **10 µIU/mL.**

SPECIFICITY (CROSS-REACTIVITY)
The specificity of the dbc prolactin ELISA kit was determined by measuring the apparent prolactin values of the following compounds:

Substance	Concentration Range	Apparent PRL Value (µIU/mL)
hCG (WHO 75/537)	100-2500 IU/L	Not Detected
FSH (WHO 1st 83/575)	25-4000 IU/L	Not Detected
hGH (WHO 80/505)	10-1000 mg/L	Not Detected
PL	0.1-50 mg/L	Not Detected
TSH (WHO 80/558)	25-1000 mIU/L	Not Detected

The specificity towards other structural forms of prolactin, including macroprolactin has not been determined.

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same calibrator curve. The results (in µIU/mL) are tabulated below:

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

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV%
1	237	18	7.6
2	589	85	14.4
3	1725	277	13.2

RECOVERY
Spiked samples were prepared by adding defined amounts of prolactin to three patient serum samples. The results
(in µIU/mL) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	55	-	-
Unspiked	95	117	81.2
+62	172	185	93.0
+130	508	597	85.1
+542			
2	59	-	-
Unspiked	127	108	117.6
+49	258	204	126.5
+145	792	834	95.0
+775			
3	707	-	-
Unspiked	807	852	94.7
+145	1356	1092	124.2
+385	1868	1482	126.0
+775			

LINEARITY
Three patient serum samples were diluted with calibrator A. The results (in µIU/mL) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	292	-	-
1:2	124	146	84.9
1:4	87	73	119.2
1:8	41	37	110.8
2	444	-	-
1:2	202	222	91.0
1:4	116	111	104.5
1:8	69	56	123.2
3	1965	-	-
1:2	1014	983	103.2
1:4	427	491	87.0
1:8	209	246	85.0

EXPECTED NORMAL VALUES
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µIU/mL)
Males	67-360
Females	55-2500
Postmenopausal	<400

REFERENCES

- Forsyth, I.A., et al., J. Endocrinol. 51:157, 1971.
- Friesen, H.G. et al., J Clin Endocrinol. 31:611, 1970.
- Friesen, H.G. et al., Nature 232:19, 1971.
- Hwang P., et al., Pro. Nat. Acad. Sci. US 68:1902, 1971.
- Hwang P., et al., J. Biol. Chem. 247:1955, 1972.
- Jacobs L.S., et al., J. Clin. Endocrinol. 34:484, 1972.
- Kleinberg D.L., et al., J. Clin Invest. 50:1557, 1971.
- Kelly, P.A., et al., Rec. Horm. Res. 40:379, 1984.
- Kelly, P.A. et al., Endocrinology 95:532, 1974.
- Lewis U.J., et al., Biochem. Biophys. Res. Commun. 44:1169, 1971.
- Linzer, D.I.H., et al., Proc. Natl. Acad. Sci. USA 81:4255, 1984.
- McLeod. R.M. Regulation of Prolactin Secretion: In Frontiers in Neuroendocrinology (Martin L. and Garrong N.F. eds) Raven Press 164, 1976.
- Nickilics R., et al., Nature 316:511, 1985.
- Rasmussen L.C., et al., Clin. Endocrinol. 26:321, 1987.
- Reyes F.I. et al., Am. J. Obstet. Gynecol. 129:557, 1977.
- Riddle, O., et al., Am. J. Physiol. 105:191, 1973.
- Shiu,R.P.C., et al., J. Biol. Chem. 260:11307, 1985.
- Allolio B, Hoeppener A, Leonhardt U, Deuss U, Winkelmann W. Size Heterogeneity of Immunoreactive Prolactin in Patients with Prolactinoma. Acta Endocrinologica 1987; 114:475-482.
- Cavaco B, Leite V, Santos MA, Sobrinho LG. Anti-prolactin (PRL) Autoantibodies Cause Asymptomatic Hyperprolactinemia: Bioassay and Clearance Studies of PRL-immunoglobulin G Complex.
- J. Clin. Endocrinol. Metab. 1995; 80:2342-2346.
- Bonhoff A, Vuille JC, Gomez F, Gellersen B. Identification of Macroprolactin in a Patient with Asymptomatic Hyperprolactinemia as a Stable PRL-IgG Complex. Exp. Clin. Endocrinol. 1995; 103:252-255.
- Fahie-Wilson M. Detection of Macroprolactin Hyperprolactinemia in Commercial Assays for Prolactin. Clin. Chem. 2000; 46:2022-2023.
- Schneider W, Marcovitz S, Al-Shammari S, Yago S, Chevalier S. Reactivity of Macroprolactin in Common Automated Immunoassays.
- Clin. Biochem. 2001;34:469-473.

Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!