BECKMAN COULTER

CE 2797

ACCESS
Immunoassay Systems

Instructions For Use

ACCESS PROLACTIN

REF 33530

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

The Access Prolactin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of prolactin (PRL) levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

Elevated PRL levels can be used to aid in the differential diagnosis of female infertility, impotence and infertility in men, and pituitary tumors.[1,2,3,4,5] In addition, measurement of PRL can be used to assess the success of dopamine-agonist therapy in treating prolactinomas.[6]

Prolactin (PRL) is a single chain polypeptide composed of 198 amino acids with three inter-chain disulfide bonds and a molecular weight of approximately 22,500 daltons.[1,7,8,9] Prolactin is secreted by the anterior cells of the pituitary gland. Prolactin secretion is controlled by the hypothalamus primarily through the release of prolactin inhibiting factor (dopamine) and prolactin releasing factor (serotonin).[2,7,9,10] Thyrotropin releasing hormone (TRH) stimulates PRL secretion and is useful as a provocative test to evaluate PRL reserves and abnormal secretion of PRL by the pituitary.[1,2,7,8,9,10]

The primary physiological function of PRL is to stimulate and maintain lactation in women.[2] In normal females, serum PRL levels generally range from 1-25 ng/mL (µg/L) while normal male levels typically range from 1-20 ng/mL (µg/L).[3,11] Normal PRL secretion varies with time which results in serum PRL levels 2-3 times higher at night than during the day.[7,8] The biological half-life of PRL is approximately 20-50 minutes.[8,11] Serum PRL levels during the menstrual cycle are variable and commonly exhibit slight elevations during the mid-cycle.[8] Prolactin levels in normal individuals tend to rise in response to physiologic stimuli including: sleep, exercise, nipple stimulation, sexual intercourse, hypoglycemia, pregnancy, and surgical stress.[2,3,7,8,9,10,11]

Prolactin is secreted by the anterior pituitary gland and is required for normal breast development and lactation in women.[4] Elevated PRL levels may be detected during the eighth week of pregnancy with levels continuing to rise throughout gestation. In the absence of breast feeding, PRL levels return to normal within three weeks after birth.[3,4]

Various drugs have been shown to either increase or decrease PRL levels. Administration of L-dopa suppresses PRL secretion.[9,10,11,12] Bromocriptine inhibits PRL secretion and has been used in the treatment of amenorrhea and galactorrhea due to hyper-prolactinemia.[1,2,4,11] Administration of psychotropic drugs (phenothiazines), anti-hypertensive drugs (reserpine), and TRH tend to increase PRL secretion.[4,10] Estrogen therapy also tends to elevate serum PRL levels.[1,2,4,9,10,11,12]

METHODOLOGY

Assay type: one-step, sandwich

The Access Prolactin assay is a simultaneous one-step immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel along with polyclonal goat anti-PRL alkaline phosphatase conjugate, and paramagnetic particles coated with mouse monoclonal anti-PRL antibody. The serum or plasma (heparin) PRL binds to the monoclonal anti-PRL on the solid phase, while the goat anti-PRL-alkaline phosphatase conjugate reacts with a different antigenic site on the serum PRL.

After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[13,14]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

REAGENTS

CONTENTS

Access Prolactin Reagent Pack

Ref. No. 33530: 100 determinations, 2 packs, 50 tests/pack

The same reagent formulation is used on all Access Immunoassay Systems.

- All antisera are polyclonal unless otherwise indicated.

Well	Contents	Ingredients
R1a:	3.25 mL	Paramagnetic particles coated with goat anti mouse IgG: mouse monoclonal anti-Prolactin complexes suspended in TRIS buffered saline, with bovine serum albumin (BSA), surfactant, < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	6.98 mL	Goat anti-Prolactin-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, surfactant, BSA with protein (goat), 0.2% sodium azide, and 0.1% ProClin 300.

*ProClin is a trademark of LANXESS Corp.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Prolactin Particles (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Prolactin Conjugate (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Sodium Azide < 0.2%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Prolactin Calibrators
   Provided at zero and approximately 2, 10, 20, 100 and 200 ng/mL (µg/L)
   Ref. No. 33535
2. Quality Control (QC) materials: commercial control material

3. Lumi-Phos PRO
   Ref. No. B96000
4. UniCel DxI Wash Buffer II
   Ref. No. A16793
5. Optional materials for dilution:
   - Access Sample Diluent A
     - Vial Ref. No. 81908
     - Diluent Pack Ref. No. A79783

REAGENT PREPARATION

Provided ready to use.

REAGENT STORAGE AND STABILITY

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	28 days

- Store upright.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.

CALIBRATION

CALIBRATION INFORMATION

An active calibration is required for all tests. Calibration is required every 28 days. See calibrator Instructions For Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials are essential for monitoring the system performance. Quality controls with varying concentration ranges should be run individually at least once every 24 hours when the assay is being performed.[15] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

TESTING PROCEDURE(S)

PROCEDURE

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

3. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
4. Use 30 µL of sample for each determination in addition to the sample container and system dead volumes. Use 50 µL of sample in addition to the sample container and system dead volumes for each determination run with the automated dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
5. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), µg/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 1. To manually convert concentrations to SI Units of mIU/L, multiply µg/L by multiplication factor 21.2.[16]

LIMITATIONS

1. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[17,18] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
2. Other potential interferences in the patient sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.[19] Carefully evaluate results if the sample is suspected of having these types of interferences.
3. The results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
4. The Access Prolactin assay has minimal interference from macroprolactin[20,21,22], as such, prolactin levels may appear higher than those levels determined with other prolactin assays.
5. Access Prolactin assay does not demonstrate any hook effect up to 30,000 ng/mL.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. Test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

MEASURING INTERVAL

Approximately 0.25 - 200 ng/mL (µg/L)

Automated dilution: Up to approximately 2,000 ng/mL (µg/L)

Samples can be accurately measured within the measuring interval defined as the lower Limit of Detection (LoD) and the highest calibrator value.

1. If a sample contains less than the lower limit for the assay, report the result as less than that value.
2. If a sample contains more than the stated value of the highest calibrator, report the result as greater than that value. Alternatively, the sample may be diluted to obtain a result.
   - For automated dilutions, the system dilutes one volume of sample with 9 volumes of Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions.

- For manual dilutions, dilute one volume of sample with 9 volumes of Access Sample Diluent A or Access Prolactin Calibrator S0. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

EXPECTED VALUES

1. Each laboratory should validate or establish its own reference intervals to assure proper representation of specific populations.
2. Prolactin levels were measured in human serum samples from 146 adult males, 121 premenopausal females (< 50 years of age), and 88 postmenopausal (≥ 50 years of age). The range of prolactin levels are summarized below:

	Males (PRL ng/mL [µg/L])	Females (PRL ng/mL [µg/L])	
		Premenopausal (< 50 years of age)	Postmenopausal (≥ 50 years of age)
Number	146	121	88
Median	5.53	8.28	6.20
Range†	2.64-13.13	3.34-26.72	2.74-19.64

†Non-parametric estimate of 95% confidence interval.

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

METHODS COMPARISON

A study based on CLSI EP09c, 3rd Edition[23] using Weighted Deming regression and Pearson's correlation compared the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (ng/mL [µg/L])	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
120	0.33 - 198	0.94	0.93 - 0.95	-0.0004	-0.484 - 0.0475	0.997

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[24] performed on the DxI 9000 Access Immunoassay Analyzer determined the assay demonstrated linearity across the measuring interval.

IMPRECISION

The assay was designed to have within-laboratory imprecision as listed below:

- ≤ 0.05 ng/mL (µg/L) SD at concentrations ≤ 0.5 ng/mL (µg/L)
- ≤ 10.0% CV at concentrations > 0.5 ng/mL (µg/L)

A study based on CLSI EP05-A3[25] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in duplicate in 2 runs per day for a minimum of 20 days.

ng/mL (µg/L)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	88	0.28	0.01	2.8	0.00	0.0	0.01	3.7	0.01	4.7
Sample 2	88	7.4	0.17	2.4	0.32	4.3	0.23	3.2	0.43	5.8
Sample 3	88	15	0.5	3.2	0.5	3.2	0.4	2.7	0.8	5.3
Sample 4	88	30	1.0	3.4	0.9	3.0	0.6	2.1	1.5	5.0
Sample 5	88	41	1.7	4.0	0.0	0.0	0.8	1.9	1.8	4.4
Sample 6	88	82	3.4	4.2	1.8	2.2	0.0	0.0	3.9	4.7
Sample 7	84	141	7.6	5.4	0.0	0.0	3.2	2.3	8.2	5.8

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 10 mg/dL bilirubin, lipemic samples containing the equivalent of 400 mg/dL cholesterol or 1,800 mg/dL triglycerides and hemolyzed samples containing up to 500 mg/dL hemoglobin do not affect the concentration of prolactin assayed. The addition of human albumin to the endogenous albumin in samples up to 5-9 g/dL (50-90 g/L) does not significantly affect the concentration of prolactin assayed.

No significant cross-reactivity was observed when rhGH, hCG, hFSH, hTSH, or hPL were added to the Access Prolactin Calibrator S1 (2 ng/mL) at 10.82 IU/L, 252,000 IU/L, 65,700 IU/L, 112 IU/L, and 10 µg/mL respectively. hLH at 122,000 IU/L gives a 0.01% cross-reactivity.

DETECTION CAPABILITY

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted on the DxI 9000 Access Immunoassay Analyzer following CLSI guideline EP17-A2.[26] The LoB study included multiple reagent lots and 3 instruments over a minimum of 3 days. The LoD and LoQ studies included multiple reagent lots and 3 instruments over a minimum of 5 days.

	Maximum Observed Result	Design Criteria
	ng/mL (µg/L)	ng/mL (µg/L)
Limit of Blank (LoB)	0.01	≤ 0.25
Limit of Detection (LoD)	0.02	≤ 0.25
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.02	≤ 0.25

ADDITIONAL INFORMATION

For a patient/user/third party in the European Union and in countries with identical regulatory regime (Regulation 2017/746/EU on In vitro Diagnostic Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorized representative and to your national authority.

The Summary of Safety and Performance is available from the EUDAMED database: ec.europa.eu/tools/eudamed

REVISION HISTORY

Revision A

New release of DxI Access Immunoassay Analyzer reagent IFU.

Revision B

Added Translations.

Revision C

Added Translations.

Revision D

Updated ProClin trademark statement.

Revision E

Added Translations.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

REFERENCES

1. Ashby, CD. Prolactin. In Methods in clinical chemistry, 258-265. Edited by Pesce, AJ, Kaplan, LA. CV Mosby, St. Louis, MO, 1987.

2. Frohman, LA. Diseases of the anterior pituitary. In Endocrinology and metabolism, 247-337. Edited by Felig, P. McGraw Hill, New York, NY, 1987.

3. Howanitz, JH. Evaluation of endocrine function. In Clinical diagnosis and management by laboratory methods, 308-348. Edited by Henry, JB. WB Saunders, Philadelphia, PA, 1991.

4. Frantz, AG. The breasts. In Textbook of endocrinology, 400-411. Edited by Williams RH. WB Saunders, Philadelphia, PA, 1981.

5. Bardin, CW. The testes. In Textbook of endocrinology, 293-354. Edited by Williams RH. WB Saunders, Philadelphia, PA, 1981.

6. N Rifai, AR Horvath, and CT Wittwer. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th Edition. Elsevier, St. Louis, Missouri (2018). In Chapter 65: Pituitary Function and Pathophysiology, WE Winter, A McCormack, and RL Bertholf. Pages 1492-1529.

7. Whitley, RJ. Endocrinology. In Tietz textbook of clinical chemistry, 1660-1694. Edited by Burtis, CA, Ashwood, ER. WB Saunders, Philadelphia, PA,1994.

8. Jeffcoate, SL. Assays for prolactin: guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem, 1986. 23:638-651.

9. Franks, S. Prolactin. In Hormones in blood, 280-331. Edited by Gray, CH, James, VHT. Academic Press, London, UK, 1979.

10. Reichlin, S. Neuroendocrinology. In Textbook of endocrinology, 589-645. Edited by Williams RH. WB Saunders, Philadelphia, PA, 1981.

11. Kohler, PO. Diseases of the hypothalamus and anterior pituitary. In Harrison's Principles of internal medicine, 587-604. Edited by Pertersdorf, RG. McGraw Hill, New York, NY, 1983.

12. Jacobs, LS. Prolactin. In Methods of hormone radioimmunoassay, 199-222. Edited by Jaffe, BM, Behrman HR : Academic Press, New York, NY, 1979.

13. World Health Organization. (2002). Use of Anticoagulants in Diagnostic Laboratory Investigations (WHO Standard No. WHO/DIL/LAB/99.1 Rev.2).

14. Approved Guideline - Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests, GP44-A4. 2010. Clinical and Laboratory Standards Institute.

15. Cembrowski GS, Carey RN. Laboratory quality management: QC ⇌ QA. ASCP Press, Chicago, IL, 1989.

16. Ellis, MJ, Livesey JH, Soule SG. Macroprolactin, big-prolactin and potential effects on the misdiagnosis of hyperprolactinemia using the Beckman Coulter Access Prolactin assay. Clinical Biochemistry 39 (2006) 1028-1034.

17. Kricka L. Interferences in immunoassays - still a threat. Clin Chem 2000; 46: 1037-1038.

18. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613-621.

19. Lingwood D, Ballantyne JS. Alkaline phosphatase-immunoglobulin conjugate binds to lipids in vitro, independent of antibody selectivity. Journal of Immunological Methods 2006; 311: 174-177.

20. Jarrige V. Macroprolactin - a possible cause of hyperprolactinaemia, misdaignosis and mistreatment. Pathology in Practice. February 2006.

21. Smith T, Suliman A, Fahie-Wilson M, McKenna T. Gross Variability in the Detection of Prolactin Sera Containing Big Big Prolactin (Macroprolactin) by Commercial Immunoassays. J Clin Endocrinol Metab. 87(12): 5410-5415; December 2002.

22. Ellis M, Reed M, Livesey J. Cross-reactivities of macroprolactin and big-prolactin in three commercial immunoassays for prolactin: A chromatographic analysis. Clinical Biochemistry 40:1285-1290; 2007.

23. Approved Guideline - Measurement Procedure Comparison and Bias Estimation Using Patient Samples, EP09c, 3rd Edition. June 2018. Clinical and Laboratory Standards Institute.

24. Approved Guideline – Evaluation of the Linearity of Quantitative Measurement Procedures, EP06-Ed2. November 2020. Clinical and Laboratory Standards Institute.

25. Approved Guideline – Evaluation of Precision of Quantitative Measurement Procedures, EP05-A3. October 2014. Clinical and Laboratory Standards Institute.

26. Approved Guideline - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, EP17-A2. June 2012. Clinical and Laboratory Standards Institute.

EC REP Beckman Coulter Ireland Inc., Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland +(353) (0) 65 683 1100

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
+(1) 800-854-3633
www.beckmancoulter.com