BECKMAN COULTER

CE 2797

ACCESS
Immunoassay Systems

Instructions For Use

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Access hLH
Luteinizing Hormone

REF 33510

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

The Access hLH assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of luteinizing hormone (LH) levels in human serum and plasma using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

The Access hLH assay is used in the aid in diagnosis of gonadal failure and pituitary failure in both males and females. It is also used in the differential diagnosis of amenorrhea, polycystic ovary disease and other causes of infertility.

Human Luteinizing Hormone (hLH, Lutropin) is made up of two non-identical, non-covalently associated glycoprotein subunits, denoted alpha and beta. It has been reported that the 28,500 dalton molecular weight hLH contains two N-linked carbohydrate chains on the alpha subunit and one asparagine-linked oligosaccharide on the beta subunit. The alpha subunit is similar in structure for the glycoproteins hLH, hCG, hFSH, and hTSH. It is the differences in the beta subunit of these glycoproteins which contributes to immunological and physiological specificity.[1,2,3]

In the female, hLH stimulates the final maturation of the follicle, follicular rupture, and ovulation.[3] Human LH is secreted by the gonadotropic cells of the anterior lobe of the pituitary gland in response to gonadotropin releasing hormone (GnRH) from the medial basal hypothalamus. Both hLH and hFSH are secreted in a pulsatile nature; however, this is less noticeable for hFSH perhaps due to the longer half life in the circulation.[3] In a normal menstrual cycle negative feedback by estradiol suppresses hLH secretion in the follicular phase. As the follicle develops (in response to hFSH) estradiol production increases which triggers an increase in GnRH and an increased sensitivity of the pituitary to GnRH. A GnRH surge results in the preovulatory (mid-cycle) surge of hLH and ovulation. Following this surge, hLH is suppressed during the luteal phase due to negative feedback from progesterone and estradiol.[3,4,5]

Variation in cycle lengths are observed in normally menstruating females due to variations in the length of the follicular phase. In the menopausal female, hLH levels are elevated in response to decreased production of ovarian estrogens and progestogens, which eliminates the negative feedback mechanism on the pituitary gland. As a result, ovulation and menstrual cycles decrease and eventually cease.[6]

In the male, hLH is often referred to as interstitial cell-stimulating hormone and influences the production of testosterone by the Leydig cells of the testes.[7]

Concentrations of hLH and hFSH are used in the differential diagnosis of amenorrhea.[8] In addition, the ratio of hLH/hFSH is used to aid in the diagnosis of polycystic ovary disease.[8] Low concentrations of hLH and hFSH may indicate pituitary failure while elevated concentrations of hLH and hFSH along with decreased concentrations of gonadal steroids may indicate gonadal failure (menopause, ovariectomy, premature ovarian syndrome, Turners Syndrome).[9]

Low concentrations of gonadotropin are usually observed in females taking oral steroid based contraceptives.[10] In the male, elevated hLH and hFSH with low concentrations of gonadal steroids may indicate testicular failure or anorchia. In Klinefelter's syndrome hLH may be elevated due to Sertoli cell failure.[11]

METHODOLOGY

Assay type: two-step, sandwich

The Access hLH assay is a sequential two-step immunoenzymatic (“sandwich”) assay. Sample is added to a reaction vessel, along with paramagnetic particles coated with goat anti-mouse: mouse anti-hLH complexes and TRIS buffered saline with protein. The hLH binds to the immobilized mouse anti-hLH on the solid phase. Materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Alkaline phosphatase conjugated goat anti-hLH is then added, which binds to the previously bound hLH on the particles.

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:[12]
   - 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.
5. Beckman Coulter, Inc. recommends that frozen specimens can be stored up to six months before testing.

REAGENTS

CONTENTS

Access hLH Reagent Pack

Ref. No. 33510: 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-hLH complexes suspended in TRIS buffered saline with bovine serum albumin (BSA), surfactant, < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	3.1 mL	TRIS-buffered saline with BSA, protein (mouse, goat), surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	3.1 mL	Goat anti-hLH-alkaline phosphatase conjugate in TRIS saline buffer with BSA, protein (goat), surfactant, < 0.1% 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

GXM/MXLH PMP (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%
Tris Buffer with Protein (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.
		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%
GXLH Conjugate (Compartment R1c)	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%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access hLH Calibrators
   Provided at zero and approximately 2, 10, 25, 100, and 250 mIU/mL (IU/L).
   Ref. No. 33515

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.[13] 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 55 µL of sample for each determination in addition to the sample container and system dead volumes. Use 105 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 mIU/mL. To change sample reporting units to the International System of Units (SI units), IU/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply mIU/mL by multiplication factor 1.

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.[14,15] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
2. Other potential interferences could be present in the sample and may cause erroneous results in immunoassays. Some examples that are documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.[16] 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.

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 0.3 - 250 mIU/mL (IU/L)

Automated dilution: Up to approximately 500 mIU/mL (IU/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 one volume 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 one volume of Access hLH Calibrator S0 or Access Sample Diluent A. 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. hLH levels were measured in human serum samples from 50 adult males, 50 postmenopausal females, and 26 normal cycling females. The cycles were synchronized to the mid-cycle hLH peak. The range of hLH levels generated at Beckman Coulter, Inc., are summarized below.

	Males hLH (mIU/mL)	Females hLH (mIU/mL)			
		Mid-Follicular Phase	Mid-Cycle Peak	Mid-Luteal Phase	Post Menopausal
Number	50	29	26	27	50
Mean	3.75	5.88	52.84	4.84	30.55
Range	1.24 - 8.62	2.12 - 10.89	19.18 - 103.03	1.20 - 12.86	10.87 - 58.64

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[17] using Passing-Bablok regression and Pearson's correlation compared the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (mIU/mL [IU/L])	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
117	0.32 - 232	1.05	1.04 - 1.06	-0.12	-0.24 - 0.022	0.99

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[18] 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:

- ≤ 0.2 mIU/mL (IU/L) SD at concentrations ≤ 2 mIU/mL (IU/L)
- ≤ 10.0% CV at concentrations > 2 mIU/mL (IU/L)

A study based on CLSI EP05-A3[19] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in duplicate in 2 runs per day for a minimum of 20 days.

mIU/mL (IU/L)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Sample 1	88	2.8	0.05	1.9	0.04	1.4	0.14	5.0	0.15	5.5
Sample 2	88	5.8	0.10	1.8	0.13	2.2	0.24	4.1	0.29	5.0
Sample 3	88	25	0.4	1.7	0.5	1.8	0.6	2.4	0.9	3.5
Sample 4	88	93	1.6	1.7	1.3	1.4	3.0	3.3	3.7	4.0
Sample 5	88	177	2.9	1.7	3.2	1.8	5.0	2.9	6.6	3.8

ANALYTICAL SPECIFICITY / INTERFERENCES

Interferents were added to samples with hLH concentrations of approximately 25 mIU/mL. Significant interference (≤10% bias) was not found for the following interferents at the indicated concentrations:

Interferent Tested	Concentration Added
Bilirubin	10 mg/dL
Hemoglobin	300 mg/dL
Triglycerides (Triolein)	1,800 mg/dL
Total protein (human serum albumin)	3 g/dL

Samples containing hLH concentrations of approximately 22 mIU/mL were spiked with hCG, hFSH, hTSH and βhLH. Significant cross reactivity (< 10% bias) was not found for the following substances at the indicated concentrations:

Substance Tested	Concentration Added
hCG	500,000 mIU/mL (IU/L)
hFSH	2,000 mIU/mL (IU/L)
hTSH	2,000 µIU/mL
βhLH	100 mIU/mL (IU/L)

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.[20] 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
	mIU/mL (IU/L)	**mIU/mL (IU/L)**
Limit of Blank (LoB)	0.02	≤ 0.2
Limit of Detection (LoD)	0.04	≤ 0.3
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.06	≤ 0.5

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.

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May be covered by one or more pat. -see www.beckmancoulter.com/patents.

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).

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