BECKMAN COULTER

CE 2797

ACCESS
Immunoassay Systems

Instructions For Use

Access hFSH
Follicle Stimulating Hormone

REF 33520

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

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

SUMMARY AND EXPLANATION

The Access hFSH 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 follicle stimulating hormone (hFSH, follitropin) is made up of two non-identical, covalently-associated glycoprotein subunits, denoted alpha and beta. It has been reported that the beta subunit of the 30,000 dalton molecular weight hFSH contains two asparagine-linked carbohydrate chains. The alpha subunit is similar in structure for the glycoproteins hFSH, hCG, hLH, and hTSH. It is differences in the beta subunit of these glycoproteins which contributes to immunological and physiological specificity.[1,2,3]

In the female, hFSH stimulates follicular growth and, in conjunction with hLH, stimulates estrogen secretion and ovulation. Following ovulation, hFSH and hLH are believed to be responsible for the transformation of the ruptured follicle into a corpus luteum and to influence the secretion of progesterone by the luteal cells.[4]

Human FSH 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 hFSH and hLH are secreted in a pulsatile nature, however, this is less noticeable for hFSH perhaps due to the longer half life of hFSH in circulation.[3] Levels of circulating hFSH vary in response to estradiol and progesterone. In a normal menstrual cycle, a slight peak of hFSH is observed toward the end of the luteal phase (most likely triggered by a fall in estradiol and progesterone which eliminates the negative feedback effect.) This begins the growth and maturation of ovarian follicles. The levels of hFSH then fall and remain low through the follicular phase (due to negative feedback from estradiol and progesterone produced by the developing follicle.) At mid-cycle GnRH triggers a rise in hFSH.

The function of this mid-cycle peak of hFSH is unknown. Following this rise, hFSH is suppressed during the luteal phase by negative feedback from estradiol. Near the end of the menstrual cycle the small hFSH rise then begins the follicular maturation of the next cycle.[3,4,5]

Variation in cycle lengths observed in the normally menstruating female are due to variations in the length of the follicular phase. In the menopausal female, hFSH levels are elevated in response to decreased production of ovarian estrogens and progesterones 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, hFSH stimulates spermatogenesis through receptors on the Sertoli cells which are present in the seminiferous tubules of the testes. While both hLH and hFSH are required for normal maturation of spermatozoa, hFSH is less sensitive to feedback inhibition by testosterone. Human FSH is thought to be regulated in part by the peptide inhibin which is produced by the Sertoli cells in males and by granulosa cells in females.[7]

Human LH and FSH levels are used in the differential diagnosis of amenorrhea.[8] In addition, the ratio of hLH/hFSH has been used to assist in the diagnosis of polycystic ovary disease.[8] Low levels of hLH and hFSH may indicate pituitary failure while elevated hLH and hFSH levels along with decreased levels of gonadal steroids may indicate gonadal failure (menopause, ovariectomy, premature ovarian syndrome, Turner's Syndrome).[9] Low gonadotropin levels are usually observed in females taking oral steroid-based contraceptives.[10] In the male, elevated hFSH and hLH with low levels of gonadal steroids may indicate testicular failure or anorchia. In Klinefelter's syndrome hFSH may be elevated due to Sertoli cell failure.[11]

METHODOLOGY

Assay type: two-step, sandwich

The Access hFSH assay is a sequential two-step immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-mouse: mouse anti-hFSH complexes and TRIS buffered saline with protein. The hFSH binds to the immobilized mouse anti-hFSH 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-hFSH is then added and binds to the previously bound hFSH 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 three months before testing.

REAGENTS

CONTENTS

Access hFSH Reagent Pack

Ref. No. 33520: 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:	3.1 mL	Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-hFSH complexes suspended in TRIS buffered saline with bovine serum albumin (BSA), surfactant, < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	13.25 mL	Goat anti-hFSH-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with protein (bovine, murine, goat), surfactant, < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	1.67 mL	TRIS buffered saline with protein (bovine, murine, goat), surfactant, < 0.1% sodium azide, 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

FSH 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%
GXFSH 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.
		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 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 hFSH Calibrators
   Provided at zero and approximately 1, 10, 50, 100, and 200 mIU/mL (IU/L).
   Ref. No. 33525
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 30 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 antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile 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 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.[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 1.2 - 200 mIU/mL (IU/L)

Automated dilution: Up to approximately 400 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 Sample Diluent A or hFSH 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. FSH levels were measured in human serum samples from 65 adult males, 50 postmenopausal females, and 26 normal cycling females. The cycles were synchronized to the mid-cycle LH peak. The range of hFSH levels are summarized below:

	Males hFSH (mIU/mL)	Females hFSH (mIU/mL)			
		Mid-Follicular Phase	Mid-Cycle Peak	Mid-Luteal Phase	Postmenopausal
Number	65	29	26	27	50
Mean	5.88	6.43	12.27	3.45	60.76
Range	1.27 - 19.26	3.85 - 8.78	4.54 - 22.51	1.79 - 5.12	16.74 - 113.59

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 Weighted Deming 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
120	1.7 - 198	1.03	1.01 - 1.04	-0.0576	-0.163 - 0.048	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:

- ≤ 0.3 mIU/mL (IU/L) SD at concentrations ≤ 3.0 mIU/mL (IU/L)
- ≤ 10.0% CV at concentrations > 3.0 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	80	1.2	0.1	N/A	0.1	N/A	0.1	N/A	0.1	N/A
Sample 2	84	7.6	0.35	4.6	0.00	0.0	0.28	3.6	0.45	5.9
Sample 3	84	21	0.8	4.1	0.5	2.5	0.7	3.3	1.2	5.8
Sample 4	84	46	2.4	5.3	0.0	0.0	1.7	3.7	3.0	6.4
Sample 5	84	70	3.7	5.3	0.0	0.0	2.0	2.9	4.2	6.0
Sample 6	86	126	6.4	5.1	0.2	0.1	2.9	2.3	7.1	5.6

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1,800 mg/dL (20.32 mmol/L) triglycerides, and hemolyzed samples containing up to 1 g/dL (10 g/L) hemoglobin do not affect the concentration of hFSH assayed. The addition of 3 g/dL (30 g/L) human serum albumin to the endogenous albumin in samples does not affect the concentration of hFSH assayed.

No significant cross-reactivity was observed when the hCG, hLH, hTSH or βFSH were added to the Access hFSH Calibrator S0 (zero) at 500,000 mIU/mL, 1,000 mIU/mL, 2,000 µIU/mL and 22.65 ng/mL respectively.

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)	0.3	≤ 1.2
Limit of Detection (LoD)	0.5	≤ 1.2
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.6	≤ 1.2

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.

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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