BECKMAN COULTER

CE 2797

ACCESS
Immunoassay Systems

Instructions For Use

Access Ultrasensitive hGH Growth Hormone

REF 33580

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

The Access Ultrasensitive hGH assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of hGH levels in human serum and plasma using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

Ultrasensitive measurements of hGH may aid in the diagnosis of disorders of the somatotropic axis including growth hormone excess (acromegaly or giantism) or deficiency.[1,2]

Human growth hormone (hGH, somatotropin) a 191-amino acid, single chain polypeptide hormone is synthesized, stored and secreted by somatotroph cells located predominantly in the lateral wings of the anterior pituitary gland.[3] Circulating hGH molecules exist predominantly as a 22-kD protein with approximately 10% released from the pituitary as a 20-kD variant lacking amino acid residues 32-46.

Serum hGH concentrations exhibit marked variation in the course of 24 hours as a result of episodic bursts or pulses of hGH secretion and prominent circadian and sleep-associated variations in hGH release.[4] Additionally, numerous environmental and metabolic factors including (but not limited to) nutrient intake, exercise, physical stress, depression, trauma, and age, influence secretion and clearance of hGH and therefore influence the concentration existing in serum.

Due to the low levels and pulsatile secretion of hGH, provocative tests are the most common method of evaluating hGH deficiency.[1,2] Provocative testing involves stimulating the pituitary somatotrophs to secrete hGH in response to a pharmacologic stimulus. Insulin, clonidine, arginine, L-dopa, growth hormone releasing hormone, and propranolol have all been used to stimulate hGH release. Failure to achieve a peak level of hGH release is indicative of insufficiency.

Growth hormone excess can be assessed by performing a suppression test on an individual with elevated hGH.[1,5] Suppression testing can be performed by creating a hyperglycemic state with glucose and measuring the change in hGH level.

METHODOLOGY

Assay type: one-step, sandwich

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

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:[6,7]
   - 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.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have 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. Thaw samples no more than two times.

REAGENTS

CONTENTS

Access Ultrasensitive hGH Reagent Pack

Ref. No. 33580: 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 anti-hGH complexes, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	2.98 mL	Goat anti-hGH alkaline phosphatase (bovine) conjugate, BSA, < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	2.98 mL	Buffered protein (BSA, mouse, goat), < 0.1% sodium azide, 0.5% 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

hGH Paramagnetic 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%
hGH Akaline Phosphatase 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%
hGH Blocking Agent (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.
		Ethoxylated alkyl alcohol 0.1 - 0.5%
		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 Ultrasensitive hGH Calibrators
   Provided at zero and approximately 0.07, 0.7, 7, 14 and 35 ng/mL (µg/L).
   Ref. No. 33585
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 56 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.[8] 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 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.

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.[9,10] 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.[11] 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 0.01 - 35 ng/mL (µg/L)

Automated dilution: Up to approximately 70 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 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 Ultrasensitive hGH Calibrator S0 (zero) 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. hGH was measured in human serum and plasma samples from apparently healthy male and female subjects using the Access Ultrasensitive hGH assay. The observed ranges of hGH concentrations are shown below for each population represented.

Reference Group	n	Median Age (years)	Age Range	Mean Dose (ng/mL)	95% Reference Interval
Female	232	43	21-73	0.568	0.010-3.607
Male	242	41	18-66	0.113	0.003-0.971

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[12] 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)	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
154	0.022 - 30	0.99	0.98 - 0.99	-0.00025	-0.0028 - 0.0023	1.00

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[13] 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:

- ≤ 0.0056 ng/mL SD at concentrations ≤ 0.07 ng/mL
- ≤ 8.0% CV at concentrations > 0.07 ng/mL

A study based on CLSI EP05-A3[14] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in duplicate in 2 runs per day for a minimum of 20 days.

Concentration (ng/mL)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	0.036	0.0013	3.6	0.0004	1.0	0.0005	1.3	0.0014	3.9
Sample 2	80	0.23	0.007	3.0	0.000	0.02	0.003	1.4	0.008	3.3
Sample 3	80	4.4	0.10	2.4	0.07	1.7	0.11	2.4	0.17	3.8
Sample 4	80	13	0.5	3.5	0.3	2.3	0.0	0.003	0.6	4.2
Sample 5	80	28	1.1	4.0	0.0	0.01	0.3	1.2	1.2	4.2

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 20 mg/dL bilirubin, lipemic samples containing up to 3,000 mg/dL triolein, hemolyzed samples containing up to 1,000 mg/dL hemoglobin, and 6 g/L albumin added to endogenous protein in the sample do not significantly affect the measurement of hGH in the Access Ultrasensitive hGH assay.

The following table describes the cross-reactivity of the assay with substances that are similar in structure to hGH. Potential cross-reactants were spiked into an hGH sample of approximately 8 ng/mL.

Substance	Analyte Added (ng/mL)	Cross-reactivity (%)
Prolactin	26,000	0.001
FSH	5,000	-0.021
TSH	5,000	0.007
Placental Lactogen	10,000	-0.028
LH	5,000	-0.003
HCG, β subunit	10,000	0.004
ACTH	20,000	0.001
20 kD hGH variant	20	-2.542

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.[15] 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	ng/mL
Limit of Blank (LoB)	0.001	≤ 0.005
Limit of Detection (LoD)	0.008	≤ 0.01
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.009	≤ 0.02

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

Updated ProClin trademark statement.

Revision D

Added Translations.

SYMBOLS KEY

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

REFERENCES

1. N Rifai, AR Horvath, and CT Wittwer, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th ed. 2018 Elsevier, St. Louis, Missouri. In Chapter 65: Pituitary Function and Pathophysiology, Winter, W.E. et al., Page 1500–1509.

2. Melmed S., Pathogenesis and Diagnosis of Growth Hormone Deficiency in Adults, N Engl J Med, 380;26 June 2019

3. Melmed S, Adashi EY, Rock JA, Rosenwaks Z. (Eds) Reproductive endocrinology, surgery, and technology, Volume 1, Lippincott-Raven, Philadelphia, Pennsylvania, 1996, page 784-800.

4. Iranmanesh A, Grisso B, Veldhuis JD. Low basal and persistent pulsatile growth hormone secretion are revealed in normal and hyposomatotropic men studied with a new ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 1994; 78: 526-535.

5. Bancos I, Algeciras-Schiminich A, Woodmansee WW, et al: Determination of nadir growth hormone concentration cutoff following oral glucose tolerance testing using the Beckman Coulter ultrasensitive growth hormone assay patients with newly diagnosed acromegaly, acromegaly in remission, and healthy subjects. Endocr Pract 2013 Jun 27:1-26

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

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

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

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

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

11. 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.

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

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

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

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

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