BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access DHEA-S
Dehydroepiandrosterone sulfate

REF A10826

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

The Access DHEA-S assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of Dehydroepiandrosterone sulfate levels in human serum and plasma using the Access Immunoassay Systems. Dehydroepiandrosterone measurements are used in the diagnosis and treatment of DHEA-secreting adrenal carcinomas.

SUMMARY AND EXPLANATION

Dehydroepiandrosterone sulfate (DHEA-S) is a steroid synthesized primarily by the adrenal gland.[1] In those tissues containing sulfatase activity, DHEA-S can be converted to free steroid DHEA. Susbsequently, DHEA and/or DHEA-S may be partially metabolized into active androgens and estrogens.[1,2]

Serum and plasma DHEA-S levels are found to be the highest of all steroids. DHEA-S levels decrease with age in both men and women after maximum levels are reached around the third decade of life.[3] The half-life for DHEA-S is approximately 8 to 10 hours as compared to the 30 to 60 minute half-lives of other androgens.[1] The long half-life of serum DHEA-S coupled with the limited diurnal variation make DHEA-S a convenient marker for the assessment of adrenal production.[4]

DHEA-S may be used in the differential diagnosis of Cushing's syndrome.[5] DHEA-S may also be used to evaluate adrenocortical diseases, such as congenital adrenal hyperplasia and adrenal tumors.[6] In hirsute female patients, increased DHEA-S levels have been associated with virilizing adrenal tumors.[7] Patients with polycystic ovary syndrome have often demonstrated elevated levels of DHEA-S, suggesting an adrenal androgen contribution to the defect in this disorder.[8,9,10]

METHODOLOGY

Assay type: one-step, competitive

The Access DHEA-S assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-rabbit: rabbit anti-DHEA-S and DHEA-S alkaline phosphatase conjugate in TRIS-buffered protein solution.

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 inversely 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 (lithium heparin, sodium heparin and EDTA) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[11]
   - 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 DHEA-S Reagent Pack

Ref. No. A10826: 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-rabbit IgG: rabbit anti DHEA-S in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	9.95 mL	DHEA-S-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA matrix, < 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

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%
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%

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access DHEA-S Calibrators
   Provided at zero and approximately 20, 50, 200, 500 and 1,000 µg/dL (0.54, 1.36, 5.43, 13.57 and 27.14 µmol/L).
   Ref. No. A10827
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 Wash Buffer II
     Ref. No. A16792
   - Normal Saline

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.[12] 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 5 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 µg/dL. To change sample reporting units to the International System of Units (SI units), µmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply µg/dL by multiplication factor 0.02714.

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.[13,14] 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.[15] 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 3.0 - 1,000 µg/dL (0.08 - 27.14 µmol/L)

Automated dilution: Up to approximately 10,000 µg/dL (271.40 µmol/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 Wash Buffer II. Refer to the appropriate system manuals and/or Help system for instructions.
   - For manual dilutions, dilute one volume of sample with 9 volumes of Wash Buffer II or Normal Saline. 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. DHEA-S was measured in human serum, heparinized plasma, and EDTA plasma samples from apparently healthy male and female subjects using the Access DHEA-S assay. The observed ranges of DHEA-S concentrations are shown below for each population represented.

Age (years)	n	Median† (µg/dL)	95% Reference Interval†† (µg/dL)
Females			
18-21	10	177	51-321
21-30	39	170	18-391
31-40	40	141	23-266
41-50	42	121	19-231
51-60	39	58	8-188
61-70	30	61	12-133
≥ 71	33	35	7-177
Males			
18-21	10	302	24-537
21-30	44	238	85-690
31-40	45	217	106-464
41-50	43	193	70-495
51-60	36	119	38-313
61-70	29	78	24-244
≥ 71	34	45	5-253

†Actual median of samples

††Based on parametric model for 2.5% to 97.5% reference 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[16] using Passing-Bablok regression and Pearson's correlation compared the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (µg/dL)	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
155	7.2 - 959	1.02	1.00 – 1.03	0.19	-1.6 – 1.4	1.00

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[17] 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:

- ≤ 2.0 µg/dL (0.05 µmol/L) SD at concentrations < 20.0 µg/dL (0.54 µmol/L)
- ≤ 10.0% CV at concentrations ≥ 20.0 µg/dL (0.54 µmol/L)

A study based on CLSI EP05-A3[18] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in triplicate in 2 runs per day for a minimum of 20 days.

Concentration (µg/dL)			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Sample 1	126	11	0.8	7.3	0.0	0.0	0.8	6.8	1.1	9.9
Sample 2	126	23	1.0	4.2	0.5	2.4	1.1	4.7	1.5	6.8
Sample 3	126	96	2.5	2.6	1.9	1.9	4.8	5.0	5.8	6.0
Sample 4	126	256	6.1	2.4	4.1	1.6	11.6	4.5	13.8	5.4
Sample 5	126	735	20.6	2.8	8.6	1.2	29.5	4.0	37.0	5.0

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 30.0 mg/dL (513 µmol/L) bilirubin, lipemic samples containing of equivalent of 1,750 mg/dL (19.7 mmol/L) triglyceride, and hemolyzed samples containing up to 1,000 mg/dL (10 g/L) hemoglobin do not affect the concentration of DHEA-S assayed. In addition, 6.0 g/dL (60.0 g/L) human albumin added to endogenous albumin in the samples does not affect the concentration of DHEA-S assayed.

The following table lists substances that are similar in structure to DHEA-S.

These substances were tested at the concentrations indicated and found to have ≤ 1% cross-reactivity. DHEA had ≤ 0.5% cross-reactivity.

Substance	Analyte Added (µg/dL)
DHEA	4000
DHEA Glucuronide	5000
Aldosterone	5000
Androstenedione	1000
Androsterone	2000
Androsterone Glucuronide	5000
Cortisol	10,000
5-dihydrotestosterone	5000
Estradiol	5000
β-Estradiol-3-SO4-17-glucuronide	5000
Estriol	5000
Estrone	5000

Substance	Analyte Added (µg/dL)
Estrone-3-SO4	5000
19 Hydroxyandrostenedione	5000
Progesterone	5000
Testosterone	2000

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.[19] 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	
	µg/dL	µmol/L	µg/dL	µmol/L
Limit of Blank (LoB)	1.3	0.036	≤ 2.0	≤ 0.05
Limit of Detection (LoD)	2.0	0.05	≤ 3.0	≤ 0.08
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	2.0	0.05	≤ 7.0	≤ 0.19

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

New release of DxI Access Immunoassay Analyzer reagent IFU.

Revision B

Updated "Intended Use" section.

Revision C

Updated ProClin trademark statement.

SYMBOLS KEY

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

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