AIA-360 Assay Specifications
ST DHEA-S Test Code 079

No.	Item	Data
1	Calib. Req	SHOW
2	Cal 1	0 µg/dL
3	Cal 2	5 µg/dL (example)
4	Cal 3	12 µg/dL (example)
5	Cal 4	60 µg/dL (example)
6	Cal 5	300 µg/dL (example)
7	Cal 6	1,200 µg/dL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	µg/dl
11	Smpl. Vol	10
12	Dil. Vol	140
13	Assay L	2.00
14	Assay H	1000.00
15	Ref. L	
16	Ref. H	
17	Decimal	2

Visible only in Test Mode

18	Test Name	#DHEA
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	6
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.00
25	Factor1 B	0.00
26	Factor2 A	1.00
27	Factor2 B	0.00
28	V. Conc	0.00
29	G Origin	0.00

AIA-900 Assay Specifications
ST DHEA-S Test Code 079

No.	Item	Data
1	CODE	079
2	ACT	1
3	ANALYTE	#DHEA
4	LOT	***Enter current lot number
5	CALIB. 1	0 µg/dL
6	CALIB. 2	5 µg/dL (example)
7	CALIB. 3	12 µg/dL (example)
8	CALIB. 4	60 µg/dL (example)
9	CALIB. 5	300 µg/dL (example)
10	CALIB. 6	1,200 µg/dL (example)
11	CALLOT L	
12	CALLOT R	
13	UNIT	µg/dl
14	DECIMAL	1
15	ASSAY L	2.0
16	ASSAY H	1000.0
17	REF L	
18	REF H	
19	RESCHED. L	2.0
20	RESCHED. H	1000.0
21	FACTOR A	1.00
22	FACTOR B	0.00
23	SMPL. VOL.	10
24	DIL. VOL.	140
25	2 REAG	0
26	CAL. CODE	079
27	CAL. NO.	6
28	CAL. MUL	3
29	CAL. EQU	6
30	CAL. CV	90
31	DIL. SP1	1
32	DIL. SP2	1
33	DIL. CAL	1
34	DIL. CNTL.	1
35	DIL. DO	5
36	DIL. AH	5
37	DIL. CALC	1
38	DIL. CODE	079
39	DIL. NAME	DHEA
40	DIL. PRTY	3
41	PRE. SPVOL	0
42	PRE. 1VOL	0
43	PRE. 2VOL	0
44	PRE.CODE	000
45	PRE.NAME	
46	PROTOCOL	1
47	SYS. F A	1.00
48	SYS. F B	0.00

AIA-2000 Assay Specifications
ST DHEA-S Test Code 079

No.	Item	Data
1	Unit	µg/dl
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	2.00
6	Reschedule high	1000.00
7	Assay range low	2.00
8	Assay range high	1000.00
9	Specimen diluent code	79
10	Specimen diluent name	DHEA
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	5
15	Default multiplier for >H	5
16	Factor A	1.00
17	Factor B	0.00
18	Calibration code	6
19	Calibrator replicates	3
20	Calibrator lot	***Enter current lot number
21	Calibrator Concentration	
22	Cal - 01	0 µg/dL
23	Cal - 02	5 µg/dL (example)
24	Cal - 03	12 µg/dL (example)
25	Cal - 04	60 µg/dL (example)
26	Cal - 05	300 µg/dL (example)
27	Cal - 06	1,200 µg/dL (example)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	90
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	10
39	Diluent volume	140
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1.00
49	Calibration Code Check	079
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

DHEA-S
ST AIA-PACK DHEA-S

Name and Intended Use

ST AIA-PACK DHEA-S is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of dehydroepiandrosterone sulfate (DHEA-S) in human serum, heparinized plasma or EDTA plasma on Tosoh AIA System Analyzers.

Summary and Explanation of Test

DHEA-S is a steroid hormone chiefly secreted from adrenal cortex. At any age, the DHEA-S level in male is higher than that in female. The DHEA-S level is the highest during his/her twenties, thereafter, the DHEA-S level decreases gradually [1-2]. DHEA-S is used for the index of various diseases of the adrenal cortex, and is especially useful for the differential diagnosis of the adrenal lesion of Cushing's syndrome. Moreover, high level DHEA-S is often observed in patients with virilism or polycystic ovary syndrome, etc [3-5].

Principle of the Assay

The ST AIA-PACK DHEA-S is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK DHEA-S test cups. DHEA-S present in the test sample is competes with enzyme-labeled DHEA-S for a limited number of binding sites on a DHEA-S-specific antibody immobilized on magnetic beads. The magnetic beads are washed to remove the unbound enzyme-labeled DHEA-S and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled DHEA-S that binds to the beads is inversely proportional to the DHEA-S concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK DHEA-S, Cat No. 0025222)

5 trays x 20 test cups (ST AIA-PACK DHEA-S, Cat. No. 0025222)

Plastic test cups containing lyophilized twelve magnetic beads coated with anti-DHEA-S rabbit polyclonal antibody and 75 µL of DHEA-S conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Materials Required But Not Provided

The following materials are required to perform DHEA-S analysis using the ST AIA-PACK DHEA-S (Cat. No. 0025222) on the Tosoh AIA System Analyzers. The following are available separately from Tosoh.

Materials		Cat. No.
AIA-SYSTEMS:		
AIA-360		0019945
AIA-2000 ST		0022100
AIA-2000(LA)		0022101
AIA-900		0022930
AIA-900 9 Tray Sorter		0022931
AIA-900 19 Tray Sorter		0022932
AIA-PACK		
AIA-PACK SUBSTRATE SET II		0020968
AIA-PACK SUBSTRATE REAGENT II / AIA-PACK SUBSTRATE RECONSTITUENT II		
ST AIA-PACK DHEA-S CALIBRATOR SET		0025322
ST AIA-PACK DHEA-S CALIBRATOR (1)	0 µg/dL	
ST AIA-PACK DHEA-S CALIBRATOR (2)	5 µg/dL (approx.)	
ST AIA-PACK DHEA-S CALIBRATOR (3)	12 µg/dL (approx.)	
ST AIA-PACK DHEA-S CALIBRATOR (4)	60 µg/dL (approx.)	
ST AIA-PACK DHEA-S CALIBRATOR (5)	300 µg/dL (approx.)	
ST AIA-PACK DHEA-S CALIBRATOR (6)	1,200 µg/dL (approx.)	
ST AIA-PACK DHEA-S SAMPLE DILUTING SOLUTION		0025522
AIA-PACK WASH CONCENTRATE		0020955
AIA-PACK DILUENT CONCENTRATE		0020956
SAMPLE CUPS		0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUPS		0020970
AIA-PACK SAMPLE TREATMENT CUPS		0020971
ADDITIONAL REQUIREMENTS:		
PIPETTE TIPS (1000/pkg.)		019215
TIP RACK (Empty)		019216
PRELOADED PIPETTE TIPS (96 tips x 50 racks)		996010
PRELOADED PIPETTE TIPS (96 tips x 5 racks)		996005

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on Tosoh materials.

Warnings and Precautions

- The ST AIA-PACK DHEA-S is intended for in vitro diagnostic use only.
- ℞ only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Test cups from different lots or different assays should not be mixed within a tray.
- Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- The ST AIA-PACK DHEA-S contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- The material derived from human origin is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
- Do not use beyond the expiration date.
- For safe waste disposal, it is recommended that each laboratory comply with established laboratory procedures and local, state, and federal regulations.
- The vial of ST AIA-PACK DHEA-S Sample Diluting Solution should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent.
- Residual sample diluting solution should not be mixed with a new vial. It must be discarded to avoid contamination.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2 - 8 °C:	
ST AIA-PACK DHEA-S	0025222
ST AIA-PACK DHEA-S CALIBRATOR SET	0025322
ST AIA-PACK DHEA-S SAMPLE DILUTING SOLUTION	0025522
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
18 - 25 °C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

After opening ST AIA-PACK DHEA-S test cups may be stored for a maximum of 4 days (4 x 24) at 18 - 25°C on-board the analyzer. When stored over night at 2 - 8 ºC, the test cups can be used for up to 12 days after opening (12 cycles of 8 hours on board and 16 hours in the refrigerator). Once opened, if stored in the refrigerator, the test cups must be used within 30 days.

The ST AIA-PACK DHEA-S CALIBRATOR SET must be kept tightly sealed and refrigerated at 2 - 8°C. After opening, the calibrators must be used within 1 day.

After opening, ST AIA-PACK DHEA-S SAMPLE DILUTING SOLUTION should be used within 7 days as long as it is opened for up to 8 hours at 18 - 25 °C in a day and the vials are kept tightly sealed and refrigerated at 2 - 8 °C immediately after use. The sample diluting solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the vials are kept tightly sealed and refrigerated immediately after use.

Reconstituted substrate solution is stable for 3 days at 18 - 25 °C or 30 days at 2 - 8 °C. Working diluent and wash solutions are stable for 30 days at 18 - 25 °C. Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum, heparinized plasma or EDTA plasma is required for the assay. Citrated plasma SHOULD NOT BE USED.

When using serum, a venous blood sample is collected aseptically without additives. Store at 18 - 25°C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain the serum specimen for assay.

When using heparinized plasma or EDTA plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.

Samples may be stored at 2 - 8 °C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20°C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to the assay, slowly bring frozen samples to 18 - 25 °C and mix gently.

The sample volume required for analysis is 10 µL.

Procedure

1) Common Reagent Preparation

1a) Substrate Solution

Bring all reagents to 18 - 25 °C before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate and mix thoroughly to dissolve the solid material.

1b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L.

1c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

2) Calibration

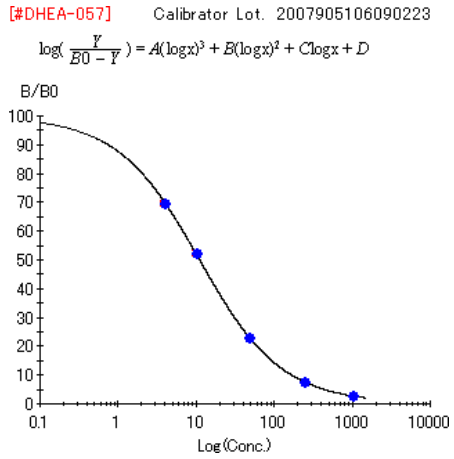
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK DHEA-S are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the ST AIA-PACK DHEA-S is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and Tosoh AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change). For further information regarding instrument operation, consult the Tosoh AIA System Operator's Manual.

A sample calibration curve from the AIA-1800 follows and show the algorithm used for calculating results.



2b) Calibration Procedure

Refer to the appropriate Tosoh AIA System Operator's Manual for the procedural instructions.

i) Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.

ii) Calibrators for ST AIA-PACK DHEA-S are provided ready for use. Tosoh recommends that all calibrators be run in triplicate.

2c) Calibration Acceptability Criteria

i) The mean rate for the CALIBRATOR (1) should be < 0.5 nM/sec.

ii) Since there is a direct relationship between concentration and rate, the rate should increase as the concentration increases.

iii) The replicate values should be within a 10% range.

2d) Calibration Review and Acceptance

i) Review the calibration curve carefully, using the criteria listed above.

ii) Edit the calibration if necessary, and then accept the calibration.

For further information regarding calibration, consult the Tosoh AIA System Operator's Manual.

3) Quality Control

3a) Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

3b) Quality Control Procedure

i) Assay quality control specimens as instructed in the Operator's Manual for the Tosoh AIA System Analyzer. In addition, refer to the Tosoh AIA System Operator's Manual for detailed instructions on defining and editing the files.

ii) Quality control should be run in accordance to local, state or federal regulations.

4) Specimen Processing

4a) Preparation

Following the specific instructions in the Operator's Manual for the Tosoh AIA System Analyzer, place samples on the instrument appropriately. Barcoded primary tubes as well as sample cups can be run on all AIA System Analyzers..

4b) Assay Procedure

i) Ensure a sufficient quantity of ST AIA-PACK DHEA-S test cups for the number of samples to be run is available.

ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA-2000 and AIA-900 will require AIA-PACK Sample Treatment Cups if onboard dilutions are utilized.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.
3. If a specimen DHEA-S concentration is found to be greater than 1,000 µg/dL, the specimen should be diluted with the ST AIA-PACK DHEA-S SAMPLE DILUTING SOLUTION and reassayed according to the Assay Procedure. The recommended dilution for specimens containing greater than 1,000 µg/dL is a 5 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 2.0 and 1,000 µg/dL. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the Tosoh AIA System Operator's Manual.
4. The Tosoh AIA System Analyzers can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK DHEA-S test cups can be used during the same run.
5. If the assay specifications for this test are not ready in the system software, the specifications must be entered under test code 079.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to DHEA-S concentration in µg/dL.

For samples requiring dilution, the AIA-2000 and AIA-900 will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programming dilutions, consult the appropriate Tosoh AIA System Operator's Manual.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples should be assayed daily.

The minimum recommendations for the frequency of running internal control material are:

1. After calibration, two levels of the internal control are run in order to accept the calibration curve.

2. The two levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

3. After daily maintenance, at least two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzer.

If one or more control value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g. symptoms, results of other tests, clinical impressions, therapy, etc.).

Using ST AIA-PACK DHEA-S, the highest measurable concentration of DHEA-S in specimens without dilution is 1,000 µg/dL, and the lowest measurable concentration is 2.0 µg/dL (assay sensitivity).

Although the approximate value of the highest calibrator is 1,200 µg/dL, the exact

concentration may be slightly different. The assay specification, ASSAY RANGE HIGH, should be defined as the upper limit of the assay range, 1,000 µg/dL.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. All results should be interpreted with respect to the clinical picture of the patient.

Heterophile antibodies are known to interfere in assays of this type. ST AIA-PACK DHEA-S has been designed to minimize the effects of heterophile antibodies, but interference from high titers cannot be ruled.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval corresponding to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.

Reference Ranges

The reference range study was developed with reference to the CLSI protocol entitled: How to Define and Determine Reference Intervals in the Clinical Laboratory (C28-A3).

The interval given here was determined in serum samples from 339 apparently healthy individuals.

Females

Age	n	Reference range (ug/dL)
<20	43	20.91 to 349.95
20 - 29	45	50.67 to 413.78
30 - 39	18	71.4 to 208.1
40 - 49	13	33.8 to 303.4
49 - 59	8	40.8 to 222.0

Males

Age	n	Reference range (ug/dL)
<20	48	35.35 to 819.22
20 - 29	50	84.04 to 570.75
30 - 39	32	105.9 to 445.1
40 - 49	54	65.7 to 488.4
49 - 59	28	77.4 to 364.8

Conversion Factors

DHEA-S concentrations in this application are in units of µg/dL. Conversion to SI units of µmol/L may be made using the following equation:

µmol DHEA-S /L = µg DHEA-S /dL × 0.02714

Performance Characteristics

The following performance characteristics were determined using Tosoh AIA-1800 Automated Immunoassay Analyzer. All Tosoh AIA System Analyzers demonstrate equivalent performance.

1) Recovery

1a) Accuracy:

Sample	Initial Value (µg/dL)	DHEA-S Added (µg/dL)	Expected Value (µg/dL)	Measured Value (µg/dL)	Percent Recovery (%)
Serum A1	47.6	94.2	141.8	131.0	92.4
	47.6	47.1	94.7	89.9	94.9
	47.6	23.5	71.1	71.4	100.3
Serum B1	267.8	94.2	362.0	361.6	99.9
	267.8	47.1	314.9	316.2	100.4
	267.8	23.5	291.4	291.1	99.9
Serum C1	468.1	316.1	784.2	758.5	96.7
	468.1	158.1	626.2	616.5	98.4
	468.1	79.0	547.2	546.6	99.9
Plasma A1	89.4	55.7	145.1	145.2	100.1
	89.4	27.9	117.2	120.1	102.5
	89.4	13.9	103.3	103.4	100.1
Plasma B1	215.3	58.9	274.2	277.9	101.4
	215.3	29.4	244.7	244.2	99.8
	215.3	14.7	230.0	228.7	99.4
Plasma C1	431.2	331.9	763.1	769.2	100.8
	431.2	166.0	597.1	588.5	98.6
	431.2	83.0	514.2	522.4	101.6

1b) Dilution:

Three serum samples and three heparinized plasma samples containing high concentrations of DHEA-S were serially diluted with ST AIA-PACK DHEA-S Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (µg/dL)	Measured Value (µg/dL)	Percent Recovery (%)
Serum A2	none	51.2	51.2	100.0
	7.5/10	39.4	38.4	102.5
	5.0/10	27.2	25.6	106.0
	2.5/10	13.6	12.8	106.1
	1.0/10	5.1	5.1	100.5
Serum B2	none	292.6	292.6	100.0
	7.5/10	219.2	219.5	99.9
	5.0/10	145.6	146.3	99.5
	2.5/10	72.3	73.2	98.8
	1.0/10	30.5	29.3	104.1
Serum C2	none	516.0	516.0	100.0
	7.5/10	377.6	387.0	97.6
	5.0/10	254.5	258.0	98.6
	2.5/10	129.1	129.0	100.1
	1.0/10	52.9	51.6	102.6
Plasma A2	none	102.3	102.3	100.0
	7.5/10	76.5	76.7	99.7
	5.0/10	50.2	51.1	98.1
	2.5/10	25.0	25.6	97.8
	1.0/10	10.5	10.2	102.5
Plasma B2	none	250.3	250.3	100.0
	7.5/10	177.4	187.7	94.5
	5.0/10	121.9	125.1	97.4
	2.5/10	60.0	62.6	95.8
	1.0/10	24.6	25.0	98.2
Plasma C2	none	451.7	451.7	100.0
	7.5/10	343.5	338.8	101.4
	5.0/10	227.5	225.8	100.7
	2.5/10	113.1	112.9	100.1
	1.0/10	51.1	45.2	113.2

1c) Linearity:

The linearity for ST AIA-PACK DHEA-S was determined, based on guidance from CLSI Protocol EP06-A. The linearity was measured on the AIA-1800 instrument and has been demonstrated to be linear from 2.0 to 1,000 µg/dL, within +/- 10% difference in this interval.

2) Precision

Within run precision was determined using six controls in a total of 20 runs. Within each run, one set of duplicates per control was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation (CV).

Intra-assay (within run) Precision

Sample	Mean (µg/dL)	Pooled Standard Deviation (µg/dL)	Coefficient of Variation (%)
Serum A3	53.6	1.76	3.3
Serum B3	298.7	10.17	3.4
Serum C3	531.6	20.37	3.8
Heparinized Plasma A3	103.3	2.95	2.8
Heparinized Plasma B3	244.8	8.37	3.4
Heparinized Plasma C3	484.2	20.95	4.3

Total precision was determined by the duplicate assay of six controls in 20 separate runs. The means of each run were used to calculate the pooled standard deviation (SD) and coefficient of variation (CV).

Total Precision

Sample	Mean (µg/dL)	Pooled Standard Deviation (µg/dL)	Coefficient of Variation (%)
Serum A3	53.6	1.87	3.5
Serum B3	298.7	10.80	3.6
Serum C3	531.6	22.56	4.2
Heparinized Plasma A3	103.3	4.14	4.0
Heparinized Plasma B3	244.8	9.09	3.7
Heparinized Plasma C3	484.2	22.68	4.7

Correlation

The methods comparison study was developed with reference to the CLSI protocol entitled: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2).

Alternate Method Comparison

The correlation between serum (x) and heparinized plasma (y) on ST AIA-PACK DHEA-S was carried out using 285 patient specimens.

Slope	0.992
y-Intercept	5.1
Correlation Coefficient	0.989
Number of Samples	285

The correlation between serum (x) and EDTA plasma (y) on ST AIA-PACK DHEA-S was carried out using 285 patient specimens.

Slope	1.02
y-Intercept	1.8
Correlation Coefficient	0.985
Number of Samples	285

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Detection: The limit of detection for ST AIA-PACK DHEA-S was determined, according to CLSI guideline EP17-A. A blank sample was measured in 60 replicates. Six low level samples were measured in each 10 replicates each. As a result, the limit of detection for ST AIA-PACK DHEA-S was estimated to be 0.62 µg/dL .

Interference

The criteria for no interference are +/- 10% (90-110%) recovery of DHEA-S of the known specimen mean concentration.

- Hemoglobin (up to 440 mg/dL), free bilirubin (up to 17 mg/dL) and conjugated bilirubin (up to 19 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by triglyceride concentration (up to 1,600 mg/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Protein, as indicated by human albumin concentration (up to 20 mg/mL added to samples from apparently healthy subjects), does not interfere with the assay.
- EDTA·2K (up to 10 mg/mL) does not interfere with the assay.
- Heparin (up to 100 U/mL) does not interfere with the assay.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

Specificity

The following substances were tested for cross-reactivity. The cross-reactivity (%) is the percentage of the compound which will be identified as DHEA-S.

If these compounds are present in the specimen at the same concentration as DHEA-S, the final result will be increased by these percentages.

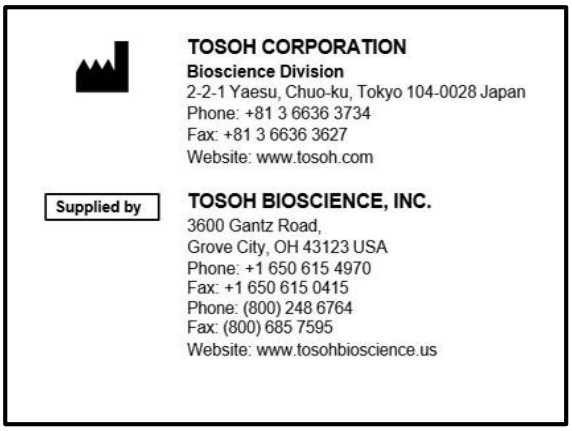
Compounds	Cross-reactivity（%）
DHEA-Sulfate	100
DHEA	N.D.
DHEA Glucuronide	0.09
Aldosterone	0.04
Androstenedione	0.17
Andorosterone	0.03
Andorosterone Glucuronide	0.10
Andorosterone Sulfate	0.30
Cortisol	0.03
5α-dihydrotestosterone	0.01
Estradiol	N.D.
β-Estradiol-3-Sulfate-17-Glucuronide	N.D.
Estriol	0.02
Estrone	N.D.
Estrone-3-Sulfate	0.09
19-hydroxyandrostenedione	0.05
Progesterone	0.05
Testosterone	0.18

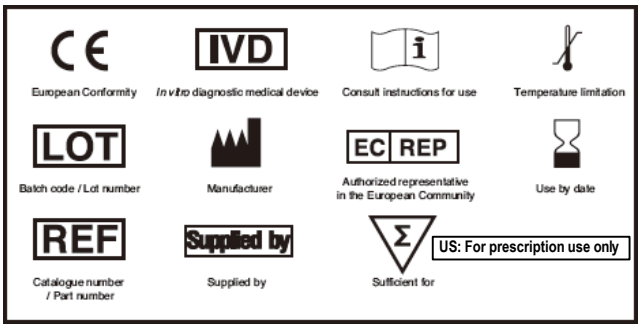
(N.D.: not detectable)

References

1. Kroboth PD, Salek FS, Pittenger AL, Fabian TJ, Frye RF : DHEA and DHEA-S, J Clin Pharmacol 39 : 327-348(1999).
2. Nestler JE, Clore JN, Blackard WG : Metabolism and Actions of Dehydroepiandrosterone in Humans. J Steroid Biochem Mol Biol 40(4-6) : 599-605(1991).
3. Yamaji T, Ishibashi M, Sekihara H, Itabashi A, Yanaihara T : Serum Dehydroepiandrosterone Sulfate in Cushing's Syndrome. J Clin Endocrinol Metab 59(6) : 1164-1168(1984).
4. Meikle AW, Daynes RA, Araneo BA. Adrenal Androgen Secretion and Biologic Effects : Endocrinol Metab Clin North Am 20(2) : 381-400(1991).

5. Chang RJ. A practical approach to the diagnosis of polycystic ovary syndrome : American Journal of obstetrics and Gynecology 191(3) : 713-717(2004).





ST AIA-PACK
DHEA-S CALIBRATOR SET

Intended Use

The ST AIA-PACK DHEA-S Calibrator Set is intended for IN VITRO diagnostic use only for the calibration of the ST AIA-PACK DHEA-S assay.

Summary and Explanation

The ST AIA-PACK DHEA-S Calibrator Set contains human sera with assigned levels of DHEA-S. DHEA-S from human origin is used in the preparation of these calibrators. Calibration should be performed according to the schedule indicated in the Tosoh AIA System Operator's Manual.

Material Provided (Cat. No. 0025322)

2 x 1 mL	ST AIA-PACK DHEA-S CALIBRATOR (1)	0.0	µg/dL

Human Serum containing no detectable concentration of DHEA-S with 0.1% sodium azide as a preservative. (ready for use)

2 x 1 mL	ST AIA-PACK DHEA-S CALIBRATOR (2)	5	µg/dL (approx.)
	ST AIA-PACK DHEA-S CALIBRATOR (3)	12	µg/dL (approx.)
	ST AIA-PACK DHEA-S CALIBRATOR (4)	60	µg/dL (approx.)
	ST AIA-PACK DHEA-S CALIBRATOR (5)	300	µg/dL (approx.)
	ST AIA-PACK DHEA-S CALIBRATOR (6)	1200	µg/dL (approx.)

Human serum containing the assigned concentration of DHEA-S (described on each vial) with 0.1% sodium azide as a preservative. (ready for use)

Warnings and Precautions

1. The ST AIA-PACK DHEA-S Calibrator Set is for in vitro diagnostic use.
2. This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.

3. The material derived from human origin used in the preparations of these calibrators has been tested by FDA-cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human origin will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
4. Do not use beyond the expiration date.

Preparation of Reagents

- The ST AIA-PACK DHEA-S Calibrators 1-6 are provided ready for use.
- Bring the calibrator to 18 - 25 °C for use.
- Always store the Calibrator Set in an upright position at 2 - 8 °C when not in use.

Storage and Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK DHEA-S Calibrator Set is stable until the expiration date on the label. Calibrator materials should be used within 24 hours after opening, provided the vials are kept tightly sealed and refrigerated at 2 - 8 °C.

Procedure

Refer to the Calibration Procedure in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the AIA System Operator's Manual.

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer software.
2. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup).
3. Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4. Tosoh recommends that all calibrators be run in triplicate.

Assignment of Values

The ST AIA-PACK DHEA-S Calibrator Set contains assigned concentrations of DHEA-S. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 2.0 to 1200 µg/dL of DHEA-S. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Results

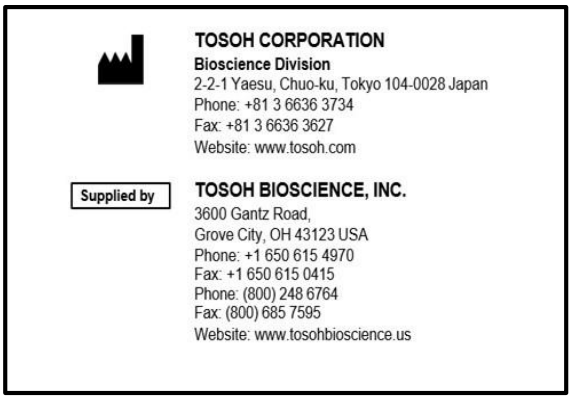
1. The mean rate for Calibrator (1) should be $<$ 0.5 nmol/(L·s).
2. Since there is an indirect relationship between concentration and rate, the rate should decrease as the concentration increases.
3. The replicate values should be within a 10 % range.

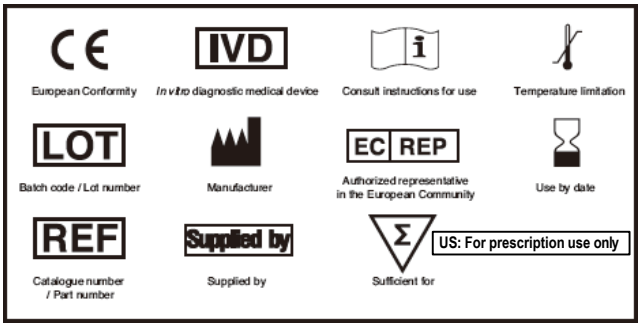
Limitations

The ST AIA-PACK DHEA-S Calibrator Set is designed solely for use with ST AIA-PACK DHEA-S assay procedures. Although the approximate value of the highest calibrator is 1200 µg/dL, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 1000 µg/dL.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.





ST AIA-PACK
DHEA-S SAMPLE DILUTING SOLUTION

Intended Use

The ST AIA-PACK DHEA-S Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples.

Summary and Explanation

The ST AIA-PACK DHEA-S Sample Diluting Solution contains a human sera with no detectable concentration of DHEA-S. This Sample Diluting Solution is to be used only with samples that are being tested for DHEA-S concentrations using the ST AIA-PACK DHEA-S assay.

Materials Provided (Cat. No. 0025522)

4 x 4 mL	ST AIA-PACK DHEA-S Sample Diluting Solution Human serum containing no detectable concentration of DHEA-S with sodium azide as a preservative.

Warnings and Precautions

- The ST AIA-PACK DHEA-S Sample Diluting Solution is intended for in vitro diagnostic use.
- This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Material derived from human origin is used for the sample diluting solution. It is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The ST AIA-PACK DHEA-S Sample Diluting Solution is provided ready for use.
- Always store the sample diluting solution upright as refrigerated at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, ST AIA-PACK DHEA-S Sample Diluting Solution is stable until the expiration date on the label. The sample diluting solution should be used within 7 days as far as it is opened for up to 7 hours at 18 - 25 °C in a day and the bottles are kept tightly sealed and refrigerated at 2 - 8 °C immediately after use. They should be used after equilibrating to 18 - 25 °C for about 30 minutes. The Sample Diluting Solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the bottles are kept tightly sealed and refrigerated immediately after use.

Procedure

Refer to the Tosoh AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. If a specimen DHEA-S concentration is found to be greater than 1000 µg/dL, the specimen should be diluted with the ST AIA-PACK DHEA-S SAMPLE DILUTING SOLUTION and assayed according to the Procedure in the insert sheet of the ST AIA-PACK DHEA-S.
2. The AIA-2000 and AIA-900 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for specimens containing greater than 1000 µg/dL is 5 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 2.0 and 1000 µg/dL.

Results

When an auto-dilution is performed, the AIA instrument will calculate the final result.

Limitations

The ST AIA-PACK DHEA-S Sample Diluting Solution is designed solely for use with ST AIA-PACK DHEA-S assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.