


**Diagnostic Automation, Inc.**




23961 Craftsman Rd, Suite E/F  
 Calabasas, CA 91302  
 Tel: (818)-591-3030, Fax: (818)-591-8383  
 Email: onestep@rapidtest.com  
 Website: www.rapidtest.com

## DHEA-S (Dehydroepiandrosterone Sulphate)

Direct Immunoenzymatic Determination of Dehydroepiandrosterone Sulphate in Serum or Plasma.

**Cat. No. 2055-17**

For in vitro diagnostic use only

<b>IVD</b>		See external label	 2°C-8°C	 Σ = 96 tests	<b>REF</b> 2055-17
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### INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Dhea-s concentration in serum and plasma

#### 1. PRINCIPLE

Dehydroepiandrosterone Sulphate (antigen) in the sample competes with horseradish peroxidase dehydroepiandrosterone sulphate (enzyme-labelled antigen) for binding onto the limited number of anti- dehydroepiandrosterone sulphate (antibody) sites on the microplates (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.

The enzyme substrate (H<sub>2</sub>O<sub>2</sub>) and the TMB-substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined.

Dehydroepiandrosterone Sulphate concentration in the sample is calculated based on a series by a set of standard.

The colour intensity is inversely proportional to the dehydroepiandrosterone sulphate concentration in the sample.

### 2. REAGENT, MATERIAL AND INSTRUMENTATION

#### 2.1 Reagent and material supplied in the kit

- Dehydroepiandrosterone Sulphate Standards
  - STD<sub>0</sub> (1 vial) 1 mL
  - STD<sub>1</sub> (1 vial) 1 mL
  - STD<sub>2</sub> (1 vial) 1 mL
  - STD<sub>3</sub> (1 vial) 1 mL
  - STD<sub>4</sub> (1 vial) 1 mL
  - STD<sub>5</sub> (1 vial) 1 mL

1. Serum diluent Conc (1 Bottle)  
 Phosphate Buffer 25 Mm ph 7.4; BSA 0.5 gr/L 20 ml

2. Conjugate (1 bottle) 12 ml  
 Dehydroepiandrosterone Sulphate-HRP conjugate

3. Coated Microplate  
 Anti-Dehydroepiandrosterone Sulphate IgG adsorbed on microplate (1 microplate breakable)

4. TMB-substrate (1 bottle) 12 ml  
 H<sub>2</sub>O<sub>2</sub>-TMB 0.25gr/L (avoid any skin contact)

5. Stop solution (1 bottle) 12 ml  
 Sulphuric acid 0.15 mol/L (avoid any skin contact)

#### 2.2 Notes

Store all reagents between +2 and + 8°C° in the dark.

Open the bag of reagent 3 (Antibody) only when it is at room temperature and close immediately after use.

Do not remove the adhesive sheets on the unused strips

#### 2.3 Reagents necessary which are not supplied with the kit

Distilled water.

#### 2.4 Auxiliary materials and instrumentation

Disposable glass tubes.

Automatic dispenser.

Microplates reader

#### 2.5 Preparation of reagents

\*. Standard (S<sub>0</sub>,S<sub>1</sub>,S<sub>2</sub>,S<sub>3</sub>,S<sub>4</sub>, S<sub>5</sub>) (liquid)

The standard has the following concentration of Dehydroepiandrosterone Sulphate:

	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>
ng/ml	0	2	8	20	80	200

#### Stability: until the expiration date printed on the kit.

The standard concentration are 50 times lower than the values reported in the reference range because in this method the samples are diluted 1/ 50 while the standards are not diluted. The concentrations to be entered in the instruments for calculations are:

	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>
µg/ml	0	0.1	0.4	1.0	4.0	10.0

When are open, the standards are stable six months at +4°C.

**Serum Diluents-** Dilute contents of serum diluents Conc. To 100 ml with distilled or deionized water in a suitable storage container. Store at room 2- 8 C° until expiration date on concentrate label.

### 3. PREPARATION OF THE SAMPLE

The determination of Dehydroepiandrosterone Sulphate can be performed in plasma as well as in serum of patients who have observed fast.

Store the sample at -20°C if the determination is not performed on the same day of the sample connection.

#### Sample dilution

Immediately before use prepare a tube for each sample and pipette:

Saline solution	mL	1
Sample	µL	20

Mix well.

### 3.1 Precaution

- Do not use heavily hemolized samples.
  - Maximum precision is required for reconstitution and dispensation of the reagents.
  - This method allows the determination of Dehydroepiandrosterone Sulphate from 0.1 µg/mL to 10 µg/mL.
- The clinical significance of the determination Dehydroepiandrosterone Sulphate can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

### 4. PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S<sub>0</sub>-S<sub>4</sub>), two for each sample, one for Blank.

Pipette:

	Standard	Sample	Blank
Diluted Sample	---	30 µl	---
Standards S <sub>0</sub> -S <sub>4</sub>	30 µl	---	---
Conjugate	100 µl	100 µl	---

Incubate at 37°C for 1hour.

Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely.

Pipette

	Standard	Sample	Blank
TMB-substrate	100µl	100µl	100µl

Incubate at room temperature (20-25°C) for 15 minutes in the dark.

Pipette:

	Standard	Sample	Blank
stop solution	100µl	100µl	100µl

Read the absorbance (E) at 450 nm against Blank.

### 5. STANDARD CURVE - CALCULATION OF RESULTS

#### 5.1 Mean absorbance and relative percentage

Calculate the mean of the absorbances (Em) for each point of the standard curve and of each sample. Express data as the percentage of the mean absorbance of B<sub>0</sub> (EmB<sub>0</sub>) with the following formula:

$$(B/B_0)\% = \frac{Em}{(Em B_0)} \times 100$$

#### 5.2 Standard curve

Plot the values of the standards expressed as (B/B<sub>0</sub>)% on the enclosed logit-log paper. Extrapolate the line passing through the points.

### 5.3 Calculation of results

Interpolate the values of the samples expressed as (B/B<sub>0</sub>)% on the standard curve to obtain the corresponding values of the concentrations expressed in µg/ml

The concentrations to be entered in the instruments for calculations are: S<sub>0</sub> S<sub>1</sub> S<sub>2</sub> S<sub>3</sub> S<sub>4</sub> S<sub>5</sub>

µg/ml 0 0.1 0.4 1.0 4.0 10.0

### 6. REFERENCE VALUES

The serum or plasma Dehydroepiandrosterone Sulphate reference values are:

	WOMAN µg/mL	MAN µg/mL
Newborns	0.9 - 1.8	0.9 - 1.8
Before puberty	0.25 - 1.0	0.25 - 1.0
Adults	0.9 - 3.6	0.9 - 3.6
After menopause	< 0.25 - 1.0	
Pregnancy	0.25 - 1.8	

### 7. PERFORMANCE CHARACTERISTICS

#### 7.1 Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

DHEA-S	90.0	%
DHEA	100.0	%
Androsterone-S-Na	48.0	%
Androstendione	20.0	%
Etiocholanolone-S-Na	0.2	%
5α Androstandione	0.01	%
Testosterone	0.01	%
Progesterone	0.01	%
17α OH-Progesterone	0.01	%
Estrone	0.01	%
Cortisol	0.001	%
Cholesterol	0.001	%

#### 7.2 Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B<sub>0</sub>, is 50 pg when the value of (B/B<sub>0</sub>)% is approx 90%.

#### 7.3 Precision

The inter and intra-run precision had a coefficient of variation of 3.2% and 5.4% respectively.

#### 7.4 Accuracy

The recovery of 2- 8- 20- 80 - 200 ng/mL of Dehydroepiandrosterone Sulphate added to "plasma-free" sample gave an average value (±SE) of 105% ± 4.8% with reference to the original concentrations.

#### 7.5 Correlation with RIA

Correlation with RIA performed on the same samples:

$$y = -0.124 + 1.127x$$

$$r = 0.996$$

$$n = 25$$

$$p < 0.001$$

### WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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