DRG® DHEA-S ELISA (Saliva) (SLV-4409)

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

Competitive immunoenzymatic colorimetric method for determination of DHEA-S concentration in saliva.
DHEA-S Saliva kit is intended for laboratory use only.

2 PRINCIPLE

DHEA-S (antigen) in the sample competes with horseradish peroxidase DHEA-S (enzyme-labelled antigen) for binding onto the limited number of anti- DHEA-S (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_2O_2) 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. DHEA-S concentration in the sample is calculated based on a series of standard.
The colour intensity is inversely proportional to the DHEA-S concentration of in the sample.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. **DHEA-S Standards** (5 vials, 1 mL each)
   STD0 - STD4
2. **Incubation Buffer** (1 vial, 30 mL)
   Phosphate buffer pH 7.5; BSA 1 g/L; Stabiliser
3. **Conjugate** (1 vial, 1 mL)
   DHEA-S-HRP conjugate
4. **Coated Microplate**, (1 microplate breakable)
   Anti-DHEA-S IgG adsorbed on microplate
5. **50X Conc. Wash Solution** (1 vial, 20 mL)
   NaCl 45 g/L; Tween-20 55 g/L
6. **TMB-Substrate** (1 vial, 15 mL)
   H_2O_2.TMB 0.26 g/L, (avoid any skin contact)
7. **Stop Solution** (1 vial, 15 mL)
   Sulphuric acid 0.15 mol/L, (avoid any skin contact)

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3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)
Saliva Collection Device (e.g. DRG SALI-TUBES (SLV-4158)

Note

Store all reagents at 2-8°C in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use. The microplate, once opened, it stable until the expiry date of kit.

4 PRECAUTIONS

- The reagents contain Proclin 300^R as preservative.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Do not use different lots of reagents.
- This method allows the determination of DHEA-S from 0.2 ng/mL to 12 ng/mL.
- The clinical significance of the determination DHEA-S can be invalidated if the specimen donor was treated with cortisone or natural or synthetic steroids.

5 PROCEDURE

5.1 Preparation of the Standard (S0,S1,S2,S3,S4)

Before use, mix for 5 min with rotating mixer.
The standard has the following concentration of DHEA-S:

	S_0	S_1	S_2	S_3	S_4
ng/mL	0	0.2	1.0	3.0	12.0

Once open stable at +4°C until the expiration date of kit.
For SI UNITS: ng/mL x 2,71 = nmol/L

5.2 Preparation of Diluted Conjugate

Prepare immediately before use.
Add 10 µL of Conjugate (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). Mix gently.
Stable 3 hours at room temperature (22÷28°C).

5.3 Preparation of Wash Solution

Dilute the content of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 mL prior to use.
For smaller volumes respect the 1:50 dilution ratio.
The diluted wash solution is stable for 30 days at 2-8°C.

5.4 Preparation of the Sample

The determination of DHEA-S can be performed in saliva.
It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw or DRG *Saliva Collection Device*.
Do not use sample collector commercially available as "SALIVETTE". Other sample collector commercially available has not been tested.

5.4.1 Method and Limitations

Collect saliva samples at the times indicated.
If no specific instructions have been given oral fluid (saliva) samples may be collected at any time for saliva collection, the following should be noted:

a. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth
b. During the day allow 1 hour after any food or drink before collecting saliva samples
c. It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

5.4.2 Saliva Processing Instructions

Let the saliva flow down through the straw into the centrifuge glass tube

1. Centrifuge the sample for 15 minutes at 3000 rpm
2. Store at – 20°C for at least 1 hour
3. Defrost samples
4. Centrifuge again for 15 minutes at 3000 rpm
5. The saliva sample is now ready to be tested.
6. Store the sample at 2-8°C for one week or at – 20°C for longer time.

5.5 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_0-S_4), two for each sample, one for Blank.

Pipette:

Reagent	Standard	Sample	Blank
Sample		50 µL	
Standards S_0-S_4	50 µL		
Dilute conjugate	150 µL	150 µL	
Incubate at 37°C for 15 minutes. Remove the contents from each well; wash the wells 3 times with 0.3 mL of diluted wash solution.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22-28°C) for 15 minutes in the dark.			
Stop solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank.			

5.6 Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

6 BIBLIOGRAPHY

1. Joshi, U. M., et al. Steroids 34 (1) 35 (1979)
2. Turkes, A., et al. J Endocrinol. 81 (2) P165 (1979)
3. Ismail A.A, et al. J.Clin.Endocr.Metab. 34,177-184 (1972)
4. Rajkowski,K.M, et al. Steroids 29 no 5 (1977)
5. Widsdom G.B. Clin. Chem. 22/8, 1243 - 1255 (1976)
6. Abraham G.E, et al. Obstet.Gynecol.,47(4),395 (1976)

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7. Abraham G.E, et al. Obstet. Gynecol.,53(1),111 (1979)
8. Hopper B.R, et al. J.Clin.Endoc.Metab.40(3),458 (1975)
9. Winter J.S.D,et al. Clin.Obste.and Gynec.,21(1),67 (1978)
10. D. Riad et al. Endocr. Reviews, 3 (4) 304 367 (1978)

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