DHEA-S Saliva

Enzyme immunoassay for the quantitative determination of DHEA-S in human saliva

Enzymimmunoassay zur quantitativen Bestimmung von DHEA-S in humanem Speichel

Only for in-vitro diagnostic use

<table>
<thead>
<tr>
<th>Language</th>
<th>Page/ Seite</th>
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<tbody>
<tr>
<td>English:</td>
<td>2 to 6</td>
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<td>Deutsch:</td>
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Product Number: DSNOV24 (96 Determinations)
1. INTRODUCTION

Dehydroepiandrosterone sulfate (DHEA-S), is a natural steroid hormone found atop of the kidneys in the human body. DHEA-S derived from enzymatic conversion of DHEA in adrenal and extradrenal tissues. DHEA-S is also produced in the gonads, adipose tissue and the brain. It is the most abundant hormone in the human body and it is precursor of all sex steroids.

As most DHEA-S is produced by the zona reticularis of the adrenal, it is argued that there is a role in the immune and stress response. DHEA-S may have more biologic roles. Its production in the brain suggests that is also has a role as a neurosteroid.

The majority of DHEA-S in saliva is non-protein bound and enters the saliva via intracellular mechanisms. Salivary DHEA-S levels are unaffected by salivary flow rate or salivary enzymes. Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels may occur in have been reported in hypoadrenalism, while elevated levels occur in several conditions, e.g. virilizing adrenal adenoma and carcinoma, 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies and in some cases of female hirsutism. Women with polycystic ovary syndrome tend to have normal or mildly elevated levels of DHEAS. As very little DHEA-S is produced by the gonads, measurement of DHEA-S levels may aid in the localization of androgen source in virilizing conditions.

DHEA-S levels show no diurnal variation.

2. INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of DHEA-S in saliva.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells are precoated with anti-DHEA-S antibodies (solid-phase). DHEA-S in the sample competes with added horseradish peroxidase labelled DHEA-S (enzyme-labelled antigen) for antibody binding. After incubation a bound/free separation is performed by solid-phase washing. The immune complex formed by enzyme-labelled antigen is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of DHEA-S in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied
- Anti-DHEA-S IgG Coated Wells: 12 breakapart 8-well snap-off strips coated with anti-DHEA-S IgG; in aluminium foil.
- Stop Solution: 1 bottle containing 15ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- DHEA-S-HRP Conjugate conc.: 1 bottle containing 1 ml horseradish peroxidase labelled DHEA-S.
- TMB Substrate Solution: 1 bottle containing 15 ml 3, 3’, 5, 5’-tetramethylbenzidine (H2O2-TMB 0.26 g/l) (avoid any skin contact).
- Incubation buffer: 1 bottle containing 30 ml phosphate buffer, pH 7.5, BSA 1 g/l, stabilizer.
- Wash Solution 50x conc: 1 bottle containing 20 ml concentrated wash solution (NaCl 45 g/l, Tween20 55 g/l)
- DHEA-S Standards: 5 bottles, 1 ml each
  - Standard 0: 0 ng/ml
  - Standard 1: 0.2 ng/ml
  - Standard 2: 1.0 ng/ml
  - Standard 3: 3.0 ng/ml
  - Standard 4: 12.0 ng/ml

4.2. Materials supplied
- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450nm
- Manual or automatic equipment for rinsing wells
- 37°C incubator
- Pipettes
- Vortex tube mixer
Distilled water
glass tubes for centrifugation and plastic straws
Timer

5. STABILITY AND STORAGE
The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION
It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

6.1. Coated snap-off Strips
The ready to use break apart snap-off strips are coated with anti-DHEA-S IgG antibodies. Store at +2…8 °C. Open the bag only when it is at room temperature. **Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date.**

6.2. DHEA-S-HRP Conjugate
Prepare immediately before use. Add 10 µl stock solution to 1 ml of incubation buffer. Mix gently. The diluted conjugate is stable for 3 h at +22 …+28°C.

6.3. DHEA-S Standards
Before use, mix for 5 min. with rotating mixer. The standards have the following concentration of DHEA-S:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>12.0</td>
</tr>
</tbody>
</table>

For SI units: ng/ml x 2.71= nmol/l

6.4. TMB Substrate Solution
The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2…8°C in the dark. **The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stable for another 6 months if stored at 2…8 °C.**

6.5. Stop Solution
The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2…8°C. **After first use stable until expiry date.**

6.6. Wash Solution
Dilute the whole content of the concentrated wash solution bottle to 1 l with distilled water in a suitable container. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days at 2…8°C.

7. SPECIMEN COLLECTION AND PREPARATION

7.1. Method and Limitations
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. **Do not use sample collector commercially available as “SALIVETTE”. Other sample collector commercially available has not been tested.**

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 has to be carried out in the morning ensure that this is carried out prior to brushing teeth.

b) During the day saliva should be collected at least 1 hour after any food or drink.

c) It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other such extraneous materials.
7.2. Processing
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.

7.3. Precaution
- Avoid the exposure of TMB substrate solution to direct sun light, metals or oxidants.
- The reagents contain Proclin 300 as preservative.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- This method allows the determination of DHEA-S from 0.2 – 12 ng/ml.
- Treatment of the patient with cortisone, natural or synthetic steroids can impair DHEA-S determination.

8. ASSAY PROCEDURE

8.1. Test Preparation
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. 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. Please allocate at least:

1 well (e.g. A1) for the substrate blank
2 wells (e.g. B1+C1) for standard 0
2 wells (e.g. D1+E1) for standard 1
2 wells (e.g. F1+G1) for standard 2
2 wells (e.g. H1+A2) for standard 3
2 wells (e.g. B2+C2) for standard 4

It is recommended to determine controls and patient samples in duplicate. Perform all assay steps in the order given and without any appreciable delays between the steps.
A clean, disposable tip should be used for dispensing each standard and each patient sample.
Adjust the incubator to 37° ± 1°C.

1. Dispense 50 µl standards and samples into their respective wells.
2. Add 150 µl diluted DHEA-S-HRP Conjugate to each well. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. Incubate for 15 min at 37 °C.
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
6. Dispense 100 µl TMB Substrate Solution into all wells.
7. Incubate for exactly 15 min at room temperature (+20 to +28 °C) in the dark.
8. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue colour developed during the incubation turns into yellow.
9. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

8.2. Measurement
Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.
If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and patient sample in the distribution and identification plan.
Where applicable calculate the mean absorbance values of all duplicates.
9. RESULTS

9.1. Calculation of results
Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (Four Parameter Logistic).
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

9.2. Reference values
As the values of salivary DHEA-S have a circadian pattern we suggest collecting the samples at the same hour (8 A.M). Each laboratory must establish its own normal ranges based on patient population.
The following value should be considered as a guideline:
Woman 0.2 – 2.5 ng/ml
Man 0.2 – 2.7 ng/ml

10. QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of DHEA-S for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra Assay Variation
Within run variation was determined by replicate determination (14x) of two different control sera in one assay. The within assay variability is ≤7.8 %.

Inter Assay Variation
Between run variation was determined by replicate measurements (9x) of three different control sera in different lots. The between assay variability is ≤14.9 %.

11.2. Specificity
The cross reaction of the antibody calculated at 50% according to Abraham:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>90%</td>
</tr>
<tr>
<td>DHEA</td>
<td>100%</td>
</tr>
<tr>
<td>Androsterone-S-Na</td>
<td>48%</td>
</tr>
<tr>
<td>Androstendione</td>
<td>20%</td>
</tr>
<tr>
<td>Etiocolanone-S-Na</td>
<td>0.2%</td>
</tr>
<tr>
<td>5-Androstendione</td>
<td>0.01%</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.01%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.01%</td>
</tr>
<tr>
<td>17 OH Progesterone</td>
<td>0.01%</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.01%</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.001%</td>
</tr>
<tr>
<td>Colesterolo</td>
<td>0.001%</td>
</tr>
</tbody>
</table>

11.3. Sensitivity
The lowest detectable concentration of DHEA-S that can be distinguished from the zero standard is 0.05 ng/ml at the 95 % confidence limit.
11.4. Accuracy
The recovery of 0.5 – 1.5 – 6.0 ng/ml of DHEA-S added to sample gave an average value (±SE) of 108.86 % ± 3.27% with reference to the original concentrations.

11.5. Method comparison
The NovaTec DHEA-S Saliva ELISA kit was compared to an analogous commercially available Kit. 31 saliva samples were analysed according in both test systems.

The linear regression curve was calculated:

\[ y = 0.37x + 1.10 \]

\[ r^2 = 0.826 \]

\[ y = \text{DHEA-S Saliva NovaTec Kit} \]
\[ x = \text{DHEA-S Saliva Salimetrics Kit} \]

12. LIMITATIONS OF THE PROCEDURE

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic 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.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

**WARNING:** In the used concentration Proclin 300® has hardly any toxicological risk upon contact with skin and mucous membranes!

**WARNING:** Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. ORDERING INFORMATION

Prod. No.: DSNOV24 DHEA-S Saliva (96 Determinations)
1. EINLEITUNG


2. VERWENDUNGSZWECK

Kompetitive immunoenzymatische kolorimetrische Method für die quantitative Bestimmung von DHEA-S in humanem Speichel.

3. TESTPRINZIP


4. MATERIAL

4.1. Mitgelieferte Reagenzien

- **Beschichtete Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit IgG Antikörpern gegen DHEA-S; in Aluminiumbeutel.
- **Stopplösung:** 1 Flasche mit 15 ml Schwefelsäure, 0,15 mol/l (Hautkontakt vermeiden).
- **DHEA-S-HRP Konjugat konz.:** 1 Flasche mit 1 ml HRP markiertem DHEA-S.
- **TMB Substrat Solution:** 1 Flasche mit 15 ml 3, 3’, 5, 5´-tetramethylbenzidine (H₂O₂-TMB 0,26 g/l) (Hautkontakt vermeiden).
- **Inkubationspuffer:** 1 Flasche mit 30 ml Phosphatpuffer, pH 7,5, BSA 1 g/l, Stabilisierer.
- **Waschlösung 50x konz.:** 1 Flasche mit 20 ml konzentrierter Waschlösung (NaCl 45 g/l, Tween20 55 g/l)
- **DHEA-S Standards:** 5 Flaschen mit je 1 ml
  - Standard 0: 0 ng/ml
  - Standard 1: 0,2 ng/ml
  - Standard 2: 1,0 ng/ml
  - Standard 3: 3,0 ng/ml
  - Standard 4: 12,0 ng/ml

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Rahmenhalter
- 1 Arbeitsanleitung
- 1 Ergebnisblatt

4.3. Erforderliche Materialien und Geräte

- 37°C Inkubator
- Photometer mit Filter 450 nm
- Manuelle oder automatische Waschvorrichtung
- Mikropipetten mit Einmalspitzen
- Vortexer
- Aqua dest.
- Zentrifugenbecher aus Glas und Plastikstrohhalme
5. STABILITÄT UND LAGERUNG

Die original verschlossenen Reagenzien sind bis zu dem auf dem Etikett angegebenen Verfalldatum haltbar, wenn sie bei +2…+8°C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Alle Reagenzien, Proben und Kontrollen sind vor ihrer Verwendung auf Raumtemperatur (20...28°C) zu bringen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit IgG Antikörpern gegen DHEA-S beschichtet. Die gebrauchsfertigen Vertiefungen sind bei 2...8°C aufzubewahren. Den Aluminiumbeutel nur öffnen, wenn er Raumtemperatur hat. Nichtverbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8°C lagern.

6.2. DHEA-S-HRP Konjugat

Unmittelbar vor Gebrauch ansetzen. 10 µl Konzentrat zu 1 ml Inkubationspuffer geben, vorsichtig mischen. Das verdünnte Konjugat ist bei +22…+28°C 3 h stabil.

6.3. DHEA-S Standards

Vor der Verwendung 5 min mit einem rotierenden Mixer mischen. Die Standards haben die folgenden DHEA-S Konzentrationen:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Konzentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>0 ng/ml</td>
</tr>
<tr>
<td>Standard 1</td>
<td>0.2 ng/ml</td>
</tr>
<tr>
<td>Standard 2</td>
<td>1.0 ng/ml</td>
</tr>
<tr>
<td>Standard 3</td>
<td>3.0 ng/ml</td>
</tr>
<tr>
<td>Standard 4</td>
<td>12.0 ng/ml</td>
</tr>
</tbody>
</table>

SI Einheiten: ng/ml x 2.71= nmol/l

6.4. TMB Substratlösung

Das Fläschchen enthält 15 ml eines Tetramethylbenzidin/Wasserstoffperoxidgemisches. Die gebrauchsfertige Lösung ist bei 2...8°C vor Licht geschützt aufzubewahren. Die Lösung ist leicht hellblau. Sollte die TMB-Substratlösung dunkelblau sein, ist sie kontaminiert und kann nicht im Test verwendet werden. Nach dem ersten

6.5. Stopplösung

Das Fläschchen enthält 15 ml 0.15 M Schwefelsäure (R36/38, S26). Die gebrauchsfertige Lösung ist bei 2...8°C aufzubewahren.

6.6 Waschlösung


7. ENTNAHME UND VORBEREITUNG DER PROBEN

Die Bestimmung von DHEA-S kann in Speichel durchgeführt werden. Es wird empfohlen, den Speichel mit einem Zentrifugenglass und einem Plastikstrohhalm zu sammeln:


Speichel zum vorgegebenen Zeitpunkt sammeln. Wenn keine besonderen Vorschriften vorliegen für die Speichelsammlung, kann diese jederzeit erfolgen. Dabei sollte folgendes beachtet werden:

a) Wenn die Speichelsammlung morgens erfolgt, sollte sichergestellt werden, dass dies vor dem Zähneputzen geschieht.
b) Während des Tages sollte zur letzten Nahrungsaufnahme (Essen und Trinken) ein Abstand von mindestens einer Stunde eingehalten werden.
c) Es ist wichtig eine klare Probe zu erhalten, d. h. es dürfen keine Kontaminationen durch z.B. Nahrung, Lippenstift, Blut enthalten sein.

7.1. Durchführung

Den Speichel durch den Strohhalm in das Zentrifugenglas laufen lassen.

1) Zentrifugieren die Probe 15 min bei 3000 rpm
2) Für mindestens 1 Stunde bei –20°C lagern
3) Proben auftauen
4) Zentrifugieren die Probe 15 min bei 3000 rpm
5) Die Speichelprobe ist nun für den ELISA Test einsetzbar.
6) Die Probe kann bei 2...8°C eine Woche gelagert werden, bei -20°C für längere Zeit.

7.3. Vorsichtsmaßnahmen
- Die Reagenzien enthalten Proclin 300®
- Das Rekonstituieren und Pipettieren der Reagenzien erfordert maximale Präzision.
- Diese Methode erlaubt die Bestimmung von DHEA-S von 0.2 – 12 ng/ml.
- TMB Substrat keinem direkten Sonnenlicht, Metall oder Oxidantien aussetzen.
- Die Behandlung mit Kortison, natürlichen oder synthetischen Steroiden kann die DHEA-S Bestimmung stören.

8. TESTDURCHFÜHRUNG

8.1. Testvorbereitung

1 Vertiefung (z.B. A1)  Blank
2 Vertiefungen (z.B B1+C1) für Standard 0
2 Vertiefungen (z.B. D1+E1) für Standard 1
2 Vertiefungen (z.B. F1+G1) für Standard 2
2 Vertiefungen (z.B. H1+A2) für Standard 3
2 Vertiefungen (z.B. B2+C2) für Standard 4

Prinzipien der Qualitätssicherung in der Laboratoriumsmedizin erfordern zur höheren Sicherheit für Kontrollen und Patientenproben mindestens Doppelbestimmungen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.
Für jeden Pipettierschritt der Kontrollen und Proben saubere Einmalspitzen verwenden.
Inkubator auf 37°C vorheizen.
1. Pipettiere 50 µl Standards und Proben in die entsprechenden Vertiefungen.
2. Gebe 150 µl DHEA-S-HRP Konjugat in jeder Vertiefung mit Ausnahme des Substratblanks.
3. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
4. Inkubiere für 15 min bei 37 °C.

Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falsch erhöhten Messergebnissen führt!
6. 100µl TMB-Substratlösung in alle Vertiefungen pipettieren.
7. Inkubiere für genau 15 min bei Raumtemperatur (22 – 28°C) im Dunkeln.

8.2. Messung
Mit Hilfe des Substratleerwertes (Blank) in A1 den Nullabgleich des Mikrotiterplatten-Photometers (ELISA-Readers) vornehmen.
Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert der Position A1 von allen anderen Extinktionswerten abgezogen werden, um einwandfreie Ergebnisse zu erzielen!
Extinktion aller Kavitäten bei 450 nm messen und die Messwerte der Kontrollen und Proben in das Ergebnisblatt eintragen.
Eine bichromatische Messung mit der Referenzwellenlänge 620 nm wird empfohlen.
Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den Mittelwert der Extinktionswerte berechnen.
9. ERGEBNISSE

9.1. Berechnung der Ergebnisse
Berechne den Mittelwert der Absorption jedes Punktes der Standardkurve und jeder Probe. Trage die Mittelwerte der Absorption gegen die Konzentration der Standards auf. Zeichne die am besten passende Kurve durch die Punkte (4 Parameter Fit).
Interpoliere die Werte der Proben anhand der Standardkurve, um die zugehörige Konzentration in ng/ml zu erhalten.

9.2. Referenzwerte
Da die Konzentration von DHEA-S in Speichel einem circadianen Rhythmus folgt wird empfohlen, die Probennahme immer zur selben Zeit durchzuführen. (8:00 vormittags).
Jedes Labor sollte die eigenen Normalwerte basierend auf der jeweiligen Patientenpopulation ermitteln. Die folgenden Werte sind nur eine Richtlinie.
Frauen 0.2 – 2.5 ng/ml
Männer 0.2 – 2.7 ng/ml

10. QUALITÄTSKONTROLLE

11. TESTMERKMALE

11.1. Präzision
Intraassay-Varianz
Die Variation innerhalb eines Testlaufs wurde ermittelt durch die wiederholte Bestimmung (14x) von zwei verschiedenen Kontrollseren in einem Test. Die Intraassay-Varianz beträgt ≤ 7.8 %.

Interassay-Varianz
Die Variation zwischen verschiedenen Testläufen (9x) wurde ermittelt durch die wiederholte Bestimmung von drei verschiedenen Kontrollseren in verschiedenen Lots. Die Interassay-Varianz beträgt ≤ 14.9 %.

11.2. Spezifität
Die Kreuzreaktion der Antikörper bei 50% berechnet nach Abraham ist:
DHEA-S 90.0 %
DHEA 100 %
Androsteron-S-Na 48.0 %
Androstendion 20.0 %
Etiocholanolon-S-Na 0.20 %
5-Androstendion 0.01 %
Testosteron 0.01 %
Progesteron 0.01 %
17 OH-Progesteron 0.01 %
Estron 0.001 %
Cortisol 0.001 %
Cholesterol 0.001 %

11.3. Analytische Sensitivität
Die niedrigste nachweisbare Konzentration an DHEA-S, die vom Standard 0 unterschieden werden kann ist 0,05 ng/ml bei einem 95% Konfidenzintervall.

11.4. Genauigkeit
0.5 – 1.5 – 6.0 ng/ml DHEA-S wurden zu einer Probe zugegeben. Die Wiederfindung lag bei durchschnittlich (± SD) bei 108,86 % ± 3,27 % in Bezug auf die Originalkonzentrationen.
11.5. Methodenvergleich
Der DHEA-S Saliva ELISA von NovaTec wurde mit einem analogen kommerziell erhältlichen Kit verglichen. Es wurden 31 Speichelproben in beiden Testsystemen untersucht. Die lineare Regressionskurve wurde berechnet:

\[ Y = 0.37x + 1.10 \]
\[ r^2 = 0.826 \]

\( y = \) DHEA-S Saliva NovaTec Kit
\( x = \) DHEA-S Saliva Salimetrics Kit

12. GRENZEN DES VERFAHRENS

13. SICHERHEITSMASSNAHMEN UND WARNHINWEISE
- Nur für in-vitro-Diagnostik.
- Reagenzien unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Die Deckel der Fläschchen nicht vertauschen um Kreuzkontaminationen zu vermeiden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat- und Standardfläschchen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten Patientenproben und Konjugat sorgfältig in die Kavitäten pipettieren.
- Den Kontakt mit Reagenzien, die Wasserstoffperoxid, Schwefelsäure und Konservierungsmittel enthalten, die beim Verschlucken toxisch sein können, vermeiden.
- Der ELISA ist nur für die Anwendung durch Fachpersonal vorgesehen, welches die Arbeitstechniken einwandfrei beherrscht.

| WARNUNG: | In der eingesetzten Proclin 300® Konzentration ist ein toxikologisches Risiko beim Kontakt mit der Haut oder Schleimhaut nahezu auszuschließen. |

13.1. Entsorgungshinweise

14. BESTELLINFORMATION
Produktnummer: DSNOV24
DHEA-S Saliva (96 Bestimmungen)
BIBLIOGRAHY/ LITERATUR

Ismail A.A et al. J. Clin. Endocr. Metab. 34, 177-184 (1972)
Rajkowski, K.M et al. Steroids 29 no 5 (1977)
# SCHEME OF THE ASSAY

## DHEA-S Saliva

### Test Preparation

- Prepare reagents and samples as described.
- Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
- Select the required number of microtiter strips or wells and insert them into the holder.

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### Assay Procedure

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Standard 0</th>
<th>Standard 1</th>
<th>Standard 2</th>
<th>Standard 3</th>
<th>Standard 4</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 0</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
</tr>
<tr>
<td>Standard 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
</tr>
<tr>
<td>Diluted conjugate</td>
<td>-</td>
<td>150 µl</td>
<td>150 µl</td>
<td>150 µl</td>
<td>150 µl</td>
<td>150 µl</td>
</tr>
</tbody>
</table>

- Cover wells with foil supplied in the kit.
- Incubate for 15 min at 37°C.
- Wash each well three times with 300 µl diluted wash solution.

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>
| Incubate for exactly 15 min. at room temperature (+22°C to +28°C) in the dark.
| Stop Solution | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| Shake the microplate gently.
| Photometric measurement at 450 nm.

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