ELISA PRODUCT INFORMATION & MANUAL

Non-species specificDHEA ELISA Kit (Colorimetric)

NBP3-07909

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Non-species specific DHEA ELISA Kit (Colorimetric)

Catalog No. NBP3-07909 480 Well (5 x 96 Well) Kit

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Description

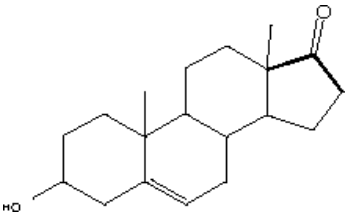
The DHEA ELISA kit is a competitive immunoassay for the quantitative determination of DHEA in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a polyclonal antibody to DHEA to bind, in a competitive manner, DHEA in the standard or sample or an alkaline phosphatase molecule which has DHEA covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of DHEA in either standards or samples. The measured optical density is used to calculate the concentration of DHEA. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

This kit measures the concentration of free, conjugated and unconjugated DHEA in biological matrices. The polyclonal antibody used in the kit has a 30% reactivity to DHEA sulfate. However, the absence of detergents or steroid analogs in the kit reagents means that protein-bound DHEA is not displaced, resulting in the measurement of free steroid only.

Introduction

Dehydroepiandrosterone (DHEA) is a 17-ketosteroid produced by side chain cleavage of 17-hydroxypregnenolone. DHEA is reversibly converted to its sulfate ester (DHEA-S) and Androstendiol. The human adrenal glands secrete both DHEA and DHEA-S which in turn serve as the precursors for both estrogenic and androgenic steroids[3]. DHEA weakly binds to corticosteroid binding globulin and albumin and strongly binds to sex hormone binding globulin[4]. DHEA is excreted in urine in its sulfate form, conjugated to glucorinide or in its free form. DHEA levels peak in early childhood and decline with age[5]. DHEA has been linked to a number of physiological conditions including depression, systemic lupus erythematosus and rheumatoid arthritis[6-8].

DHEA



Precautions

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1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.

4. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
5. The DHEA Standard provided, Catalog No. 80-0980, is supplied in ethanolic buffer at a pH optimized to maintain DHEA integrity. Care should be taken handling this material because of the known and unknown effects of steroids.

Materials Supplied

1. **Goat anti-Rabbit IgG Plates, Five Plates of 96 Wells**
   Plates using break-apart strips coated with goat antibody specific to rabbit IgG.
2. **DHEA ELISA Conjugate, 25 mL**
   A blue solution of alkaline phosphatase conjugated with DHEA.
3. **DHEA ELISA Antibody, 25 mL**
   A yellow solution of a rabbit polyclonal antibody to DHEA.
4. **Assay Buffer Concentrate, 27 mL**
   Tris buffered saline containing proteins and sodium azide as a preservative.
5. **Wash Buffer Concentrate, 100 mL**
   Tris buffered saline containing detergents.
6. **DHEA Standard, 3 x 0.5 mL**
   A solution of 500,000 pg/mL DHEA.
7. **pNpp Substrate, 100 mL**
   A solution of p-nitrophenyl phosphate in buffer. Ready to use.
8. **Stop Solution, 27 mL**
   A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic.**
9. **DHEA Assay Layout Sheet, 1 each**
10. **Plate Sealer, 10 each**

Storage

All components of this kit are stable at 4 °C until the kit's expiration date.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipets for dispensing 50 µL and 200 µL.
4. Disposable beaker for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. A 37 °C Incubator.
9. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

Sample Handling

The DHEA ELISA is compatible with DHEA samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions. However, the end user **must verify** that the recommended dilutions are appropriate for their samples. **Samples containing rabbit IgG may interfere with the assay.**

Urine samples should be centrifuged or filtered prior to use in the assay. Samples in the majority of Tissue Culture Media can also be read in the assay, provided the standards have been diluted into the Tissue Culture Media instead of Assay Buffer. There will be a small change in the binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of DHEA in the appropriate matrix. Some samples may have very low levels of DHEA present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

Materials Needed

1. DHEA Standard to allow extraction efficiency to be accurately determined.
2. ACS Grade Ethyl Acetate.
3. Glass test tubes.

Procedure

1. Add sufficient DHEA to a typical sample for determination of extraction efficiency.
2. In a fume hood add 1 mL of Ethyl Acetate for every mL of sample. Stopper and shake sample.
3. Allow layers to separate. Carefully pipet off the top organic layer and place in a clean test tube.
4. Repeat steps 1 and 2 twice more, combining the organic layers.
5. Evaporate the ether to dryness under nitrogen.
6. Dissolve the extracted DHEA with at least 250 µL of Assay Buffer by vortexing well then allow to sit for five minutes at room temperature. Repeat twice more.
7. Run the reconstituted samples in the assay immediately or keep the dried samples frozen below -20 °C in desiccation.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
9. **Prior to addition of substrate, ensure that there is no residual wash buffer in wells. Any remaining wash buffer may cause variation in assay results.**
10. This assay may also be run using an overnight format for the primary incubation (see #8 in Assay Procedure).

Reagent Preparation

1. Assay Buffer

Prepare the Assay Buffer by diluting 10 mL of the supplied concentrate with 90 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

2. DHEA Standard

Allow the 500,000 pg/mL DHEA standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 1,000 µL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 750 µL of standard diluent into tubes #2 through #7. Remove 100 µL of d iluent from tube #1. Add 100 µL of the 500,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #7.

The concentration of DHEA in tubes #1 through #7 will be 50,000, 12,500, 3,125, 781.25, 195.31, 48.83 and 12.21 pg/mL respectively. See the DHEA Assay Layout Sheet for dilution details.

Diluted standards should be used within 60 minutes of preparation.

3. Conjugate 1:10 Dilution for Total Activity Measurement

Prepare the Conjugate 1:10 Dilution by diluting 50 µL of the supplied Conjugate with 450 µL of Assay Buffer. The dilution should be used within 3 hours of preparation. **This 1:10 dilution is intended for use in the Total Activity wells ONLY**.

4. Wash Buffer

Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.

2. Pipet 100 µL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.

3. Pipet 100 µL of standards #1 through #7 into the appropriate wells.

4. Pipet 100 µL of the Samples into the appropriate wells.

5. Pipet 50 µL of Assay Buffer into the NSB wells.

6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.

7. Pipet 50 µL of the yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

8. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm or overnight at 4 °C. The plate must be covered with the plate sealer provided if the overnight incubation is to be done.

9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 washes.

10. After the final wash, empty or aspirate the wells, and firmly tap the plate dry on a lint free paper towel to remove any remaining wash buffer.

11. Add 5 µL of the blue Conjugate 1:10 Dilution (see step 3, Reagent Preparation, page 5) to the TA wells.

12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at 37 °C for 3 hours without shaking. The plate must be covered with the plate sealer provided.

13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.

14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of DHEA in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If this sort of data reduction software is not readily available, the concentration of DHEA can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   Average Net OD = Average Bound OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   $$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot Percent Bound versus Concentration of DHEA for the standards. Approximate a straight line through the points. The concentration of DHEA in the unknowns can be determined by interpolation.

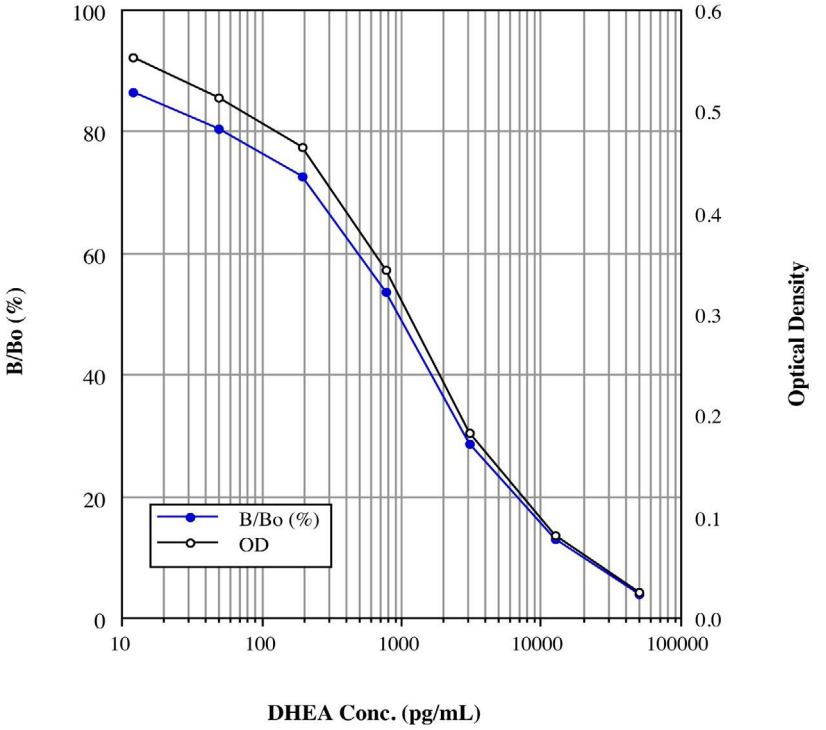
Typical Results

The results shown below, using the two hour primary incubation, are for illustration only and **should not** be used to calculate results from another assay.

Sample	Mean OD (-Blank)	Average NetOD	Percent Bound	**DHEA (pg/mL)**
Blank OD	(0.138)			
TA	2.208			
NSB	0.010	0.000	0.0	
Bo	0.650	0.640	100%	**0**
S1	0.035	0.025	3.8%	**50,000**
S2	0.092	0.082	12.8%	**12,500**
S3	0.193	0.183	28.5%	**3,125**
S4	0.354	0.344	53.7%	**781.25**
S5	0.474	0.464	72.5%	**195.31**
S6	0.524	0.514	80.3%	**48.83**
S7	0.563	0.553	86.4%	**12.21**
Unknown 1	0.097	0.087	13.6%	**10,721**
Unknown 2	0.170	0.160	25.0%	**4,187**

Typical Standard Curve

A typical standard curve using the two hour primary incubation is shown below. This curve **must not** be used to calculate DHEA concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	2.208 x 10x10 = 220.8
%NSB		0.00%
%Bo/TA	=	0.29%
Quality of Fit	=	0.9999 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	6,021 pg/mL
50% Intercept	=	938 pg/mL
80% Intercept	=	70 pg/mL

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[9].

Sensitivity

Sensitivity was calculated in Assay Buffer using the two hour primary incubation by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of DHEA measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo =	0.694 ± 0.012 (1.7%)
Average Optical Density for Standard #7 =	0.593 ± 0.020 (3.3%)
Delta Optical Density (0-12.21 pg/mL) = 0.694-0.593 =	0.101
2 SD's of the Zero Standard = 2 x 0.012 =	0.024
Sensitivity = $\frac{0.024}{0.101}$ x 12.21 pg/mL =	**2.90 pg/mL**

Linearity

A sample containing 4,195 pg/mL DHEA was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual DHEA concentration versus measured DHEA concentration.

The line obtained had a slope of 0.973 and a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of DHEA and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of DHEA in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of DHEA determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	DHEA (pg/mL)	Intra-assay %CV	Inter-assay %CV
Low	533	6.4	
Medium	1,039	5.8	
High	2,837	4.8	
Low	580		8.4
Medium	1,093		8.8
High	3,336		6.5

Cross Reactivity

The cross reactivities for a number of related steroid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 1,000,000 to 1 pg/mL. These samples were then measured in the DHEA assay, and the measured DHEA concentration at 50% B/Bo was calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
DHEA	100%
DHEA sulfate	30%
Androstenedione	0.72%
Androsterone	0.29%
Pregnenolone	0.28%
Testosterone	0.10%
Progesterone	0.06%
Estradiol	0.04%
20α-hydroxycholesterol	<0.02%
Androsterone sulphate	<0.02%
DHEA sodium sulfate	<0.02%
17α-hydroxyepipregnanolone	<0.02%
19-hydroxyandrostendione	<0.02%
17α-hydroxyprogesterone	<0.02%
Cortisol	<0.02%

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation. DHEAconcentrations were measured in a variety of different samples including tissue culture media, human saliva, urine, serum and heparin plasma. DHEA was spiked into the undiluted samples of these media which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Tissue Culture Media	95.2	Neat
Human Saliva	109.8	1:20
Human Urine	108.4	1:32
Human Serum	98.5	≥1:4
Human heparin Plasma	100.4	≥1:8

* See Sample Handling instructions on page 4 for details.

References

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