



DHEA-S ELISA Kit

Item No. 502600

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
401169	DHEA-S ELISA Monoclonal Antibody	1 vial/100 dtn	-20°C
401168	DHEA-S AChE Tracer	1 vial/100 dtn	-20°C
401167	DHEA-S ELISA Standard	1 vial/0.5 ml	-20°C
400009/ 400008	Goat Anti-Mouse IgG-Coated Plate	1 plate	4°C
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400035	Polysorbate 20	1 vial/3 ml	RT
400040	ELISA Tracer Dye	1 ea	RT
400042	ELISA Antiserum Dye	1 ea	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's DHEA-S ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 405-420 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 13).

INTRODUCTION

Background

Dehydroepiandrosterone sulfate (DHEA-S) is a major secretory product of the adrenal glands and the predominant circulating sex steroid precursor in humans.^{1,2} It is formed *via* sulfonation of DHEA by the sulfotransferase (SULT) isoform SULT2A1, also known as DHEA sulfotransferase.¹ In contrast to DHEA, DHEA-S does not show diurnal variation, is more stable with a longer half-life (7-10 hours *versus* 15-30 minutes, respectively), and is found at a higher concentration in human/primate plasma (approximately 10 μ M *versus* 10 nM, respectively).³ It reaches its peak concentration in young adulthood and decreases with age.^{3,4} DHEA-S plays an important role in maintaining pregnancy, where it acts as a precursor for placental estrogen biosynthesis.^{1,5} DHEA-S serum levels are dysregulated in several adrenal disorders, including steroid 21-hydroxylase deficiency, premature adrenarche, polycystic ovarian syndrome (PCOS), and Cushing syndrome.⁶ Alteration of baseline DHEA-S levels is also found in Crohn's disease, cardiovascular disease, chronic urticaria, and neuropsychiatric diseases.^{7,8}

About This Assay

Cayman's DHEA-S ELISA Kit is a competitive assay that can be used for the quantification of DHEA-S in plasma, serum, urine, saliva, cell culture supernatants, and other sample matrices. The assay has a range of 31.3-4,000 pg/ml, an average sensitivity (80% B/B₀) of 96 pg/ml, and a lower limit of detection (LLOD) of 30 pg/ml.

Principle of the Assay

This assay is based on the competition between free DHEA-S and a DHEA-S acetylcholinesterase conjugate (DHEA-S AChE Tracer) for a limited number of DHEA-S monoclonal antibody binding sites. Because the concentration of the DHEA-S AChE Tracer is held constant while the concentration of free DHEA-S varies, the amount of DHEA-S AChE Tracer that is able to bind to the DHEA-S Monoclonal Antibody will be inversely proportional to the concentration of free DHEA-S in the well. This antibody-DHEA-S complex binds to goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and Ellman's Reagent (which contains the substrate for AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of DHEA-S AChE Tracer bound to the well, which is inversely proportional to the amount of free DHEA-S present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{bound DHEA-S AChE tracer}] \propto 1/[\text{DHEA-S}]$$

A schematic of this process is shown in Figure 1, on page 9

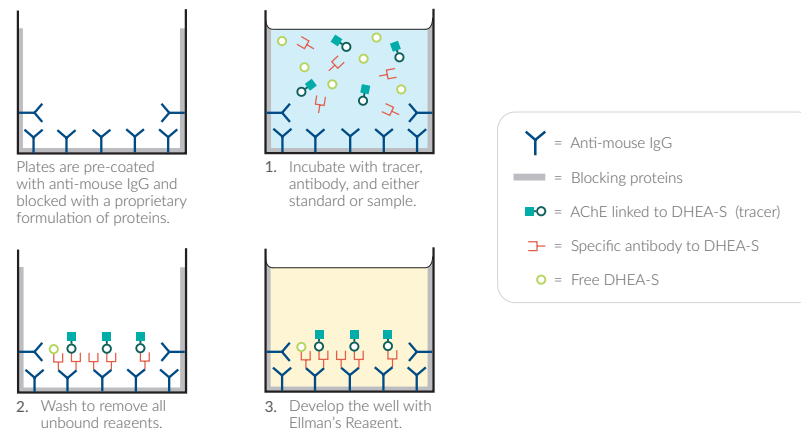


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

Total Activity (TA): total enzymatic activity of the DHEA-S AChE-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B₀ (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B₀ (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B₀) wells.

Standard Curve: a plot of the %B/B₀ values versus concentration of a series of wells containing various known amounts of analyte.

Dtn (Determination): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the midpoint (50% B/B₀) value of the tested molecule to the midpoint (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\% \text{ B/B}_0 \text{ value for the primary analyte}}{50\% \text{ B/B}_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for two months. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.*

1. Assay Buffer Preparation

Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. Wash Buffer (1X) Preparation

Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

Testing for Interference

This assay has been validated in plasma, serum, urine, saliva, and cell culture supernatants in a complete DMEM medium. Purification of these samples is not necessary. Typically, human and monkey plasma, serum, and urine must be diluted 250-2,000-fold with Assay Buffer to fall within the range of the standard curve. Saliva may need to be diluted at least two-fold. No interference of DMEM complete medium with the assay was observed.

Other sample types should be tested for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, test one or two samples at a range of dilutions, then select at least two different dilutions of each sample within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated DHEA-S concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the user and tested for compatibility in the assay.

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes. Transfer the top plasma layer into a clean test tube without disturbing the white buffy layer. Samples should be assayed immediately after collection or stored at -80°C. Dilute human and monkey plasma 250-2,000-fold with the Assay Buffer before testing.

Serum

Collect blood in vacutainers without an anticoagulant. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes. Transfer the serum layer into a clean test tube. Samples should be assayed immediately after collection or stored at -80°C. Dilute human and monkey serum 250-2,000-fold with the Assay Buffer before testing.

Urine

Urine samples should be assayed immediately or stored at -20°C or -80°C after collection. Interference in urine is infrequent. Dilute human or monkey urine 250-2,000-fold with the Assay Buffer before testing. It is recommended that the DHEA-S values in urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701) or a similar assay.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C or -20°C.
- Samples of mouse origin may contain antibodies that interfere with the assay by binding to the goat anti-mouse IgG plate. We recommend that all mouse samples be purified prior to use in the assay.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the DHEA-S ELISA Standard (Item No. 401167) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl of ultrapure water. The concentration of this solution (the bulk standard) will be 60 ng/ml.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 840 µl of Assay Buffer (1X) to tube #1 and 450 µl of Assay Buffer (1X) to tubes #2-8. Transfer 60 µl of the bulk standard (60 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 450 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 450 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should be used within two hours.

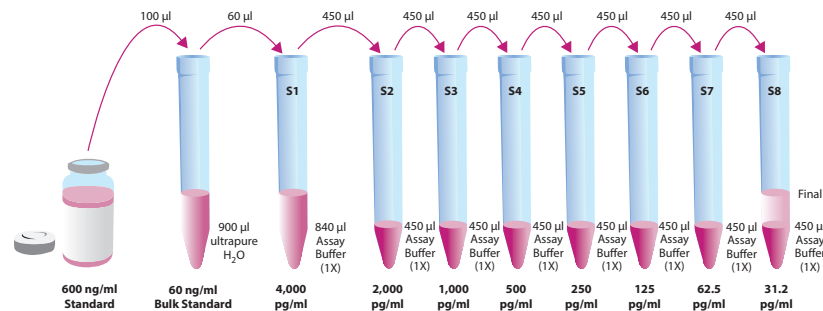


Figure 2. Preparation of the DHEA-S standards

DHEA-S Tracer

Reconstitute the DHEA-S AChE Tracer (Item No. 401168) with 6 ml of Assay Buffer (1X). Store the reconstituted DHEA-S AChE tracer at 4°C (do not freeze!). It will be stable for four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer). *NOTE: Do not store tracer with dye.*

DHEA-S ELISA Monoclonal Antibody

Reconstitute the DHEA-S ELISA Monoclonal Antibody (Item No. 401169) with 6 ml of Assay Buffer (1X). Store the reconstituted DHEA-S ELISA monoclonal antibody at 4°C (do not freeze!). It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the reconstituted antibody at a final dilution of 1:100 (add 60 µl of dye to 6 ml antibody). *NOTE: Do not store antibody with dye.*

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B₀ wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, assaying the samples in triplicate is recommended.

A suggested plate format is shown in Figure 3, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 21 for more details). It is suggested that the contents of each well be recorded on the template sheet provided (see page 29).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	5	9	13	17	21	25	29	33
B	Blk	S2	S2	1	5	9	13	17	21	25	29	33
C	NSB	S3	S3	2	6	10	14	18	22	26	30	34
D	NSB	S4	S4	2	6	10	14	18	22	26	30	34
E	B ₀	S5	S5	3	7	11	15	19	23	27	31	35
F	B ₀	S6	S6	3	7	11	15	19	23	27	31	35
G	B ₀	S7	S7	4	8	12	16	20	24	28	32	36
H	TA	S8	S8	4	8	12	16	20	24	28	32	36

- Blk = Blank Wells
- TA = Total Activity Well
- NSB = Non-Specific Binding Wells
- B₀ = Maximum Binding Wells
- S1-S8 = Standard Wells
- 1-36 = Sample Wells

Figure 3. Sample plate format

Performing the Assay

Equilibrate all reagents at room temperature prior to addition to the plate.

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. Assay Buffer

Add 100 µl of Assay Buffer (1X) to NSB wells. Add 50 µl of Assay Buffer (1X) to B₀ wells.

2. DHEA-S ELISA Standard

Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. Samples

Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed at least in duplicate (triplicate recommended).

4. DHEA-S AChE Tracer

Add 50 µl to each well except the TA and Blk wells.

5. DHEA-S ELISA Monoclonal Antibody

Add 50 µl to each well, except the TA, NSB, and Blk wells, within 15 minutes of addition of the tracer

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate two hours at room temperature on an orbital shaker.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use. Reconstitute the 100 dtn vial with 20 ml of ultrapure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays be run on different days.

2. Empty the wells and rinse five times with ~300 µl of Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of the reconstituted tracer to the TA wells.
5. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B₀ wells ≥0.6 A.U. (Blk subtracted)) in 60 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the cover sheet being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (Blk subtracted). The plate should be read when the absorbance of the B_0 wells is in the range of 0.3-2.0 A.U. (Blk subtracted). If the absorbance of the wells exceeds 2.0 A.U., wash the plate, add fresh Ellman's Reagent, and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either $\%B/B_0$ versus log concentration using a four-parameter logistic fit or as $\text{logit } B/B_0$ versus log concentration using a linear fit. *NOTE: Cayman Chemical has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. To obtain $\%B/B_0$ for a logistic four-parameter fit, multiply these values by 100.

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus DHEA-S concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B₀ in this calculation.*

$$\text{logit (B/B}_0\text{)} = \ln [\text{B/B}_0\text{}/(1 - \text{B/B}_0\text{)}]$$

Plot the data as logit (B/B₀) versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B₀ (or %B/B₀) value for each sample. Determine the concentration of each sample by identifying the %B/B₀ on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any dilution of the sample prior to the addition to the well.* Samples with %B/B₀ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

Note: If there is an error in the B₀ wells it is possible to calculate sample concentrations by plotting the corrected absorbance values and back calculating sample absorbance off the standard curve.

Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data on page 23 to determine the values of your samples.

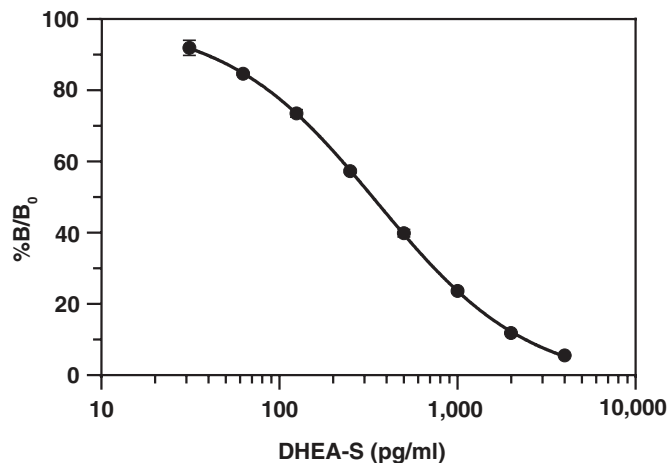
Absorbance at 414 nm (60 minutes)

Representative Data					
DHEA-S Standards (pg/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B ₀	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.000	--	--	--	--
B ₀	1.275	1.275	--	--	--
TA	1.318	--	--	--	--
4,000	0.071	0.071	5.6	5.5	7.6
2,000	0.151	0.151	11.8	4.5	5.7
1,000	0.302	0.302	23.7	3.9	3.9
500	0.508	0.508	39.8	5.5	1.1
250	0.730	0.730	57.3	8.8	5.4
125	0.937	0.937	73.5	10.0	12.5
62.5	1.079	1.079	84.6	11.5	12.7
31.3	1.171	1.171	91.8	45.8**	44.4**

Table 1. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

**Evaluate data in this range with caution



Assay Range = 31.3-4,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 87 pg/ml
Mid-point (defined as 50% B/B₀) = 334 pg/ml
Lower Limit of Detection (LLOD) = 30 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted in Assay Buffer (1X).

Figure 4. Typical standard curve

Cross Reactivity:

Compound	Cross Reactivity
DHEA-S	100%
17β-Estradiol 3-Sulfate	0.31%
Estrone sulfate	0.20%
DHEA	0.16%
Androstenediol	0.04%
Testosterone	0.01%
Pregnenolone	0.01%
Progesterone	0.01%
Dihydrotestosterone	<0.01%
Androstenedione	<0.01%
Aldosterone	<0.01%
Androsterone 3-sulfate	<0.01%
Epiandrosterone	<0.01%
17α-hydroxy Pregnenolone	<0.01%
17α-hydroxy Progesterone	<0.01%
17β-Estradiol	<0.01%
Cholesterol sulfate	<0.01%

Table 2. Cross reactivity of the DHEA-S ELISA Kit

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water B. Poor pipetting/technique
High NSB (> 10% of B ₀)	A. Poor washing B. Exposure of NSB wells to specific antibody
Very Low B ₀	A. Trace organic contaminants in the water B. Dilution error in preparing reagents
Low sensitivity (shift in dose-response curve)	A. Standard is degraded or contaminated B. Dilution error in preparing reagents
Analysis of two dilutions of a biological sample do not agree (i.e., more than 20% difference)	Interfering substances are present
Low signal in the sample wells (below the range of the standard curve)	A. AChE inhibitors are present; ensure that the samples and buffers are free of AChE inhibitors. B. Sample requires further dilution
Only TA wells develop	A. Trace organic contaminants in the water B. The tracer was not added to the wells

References

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Assay Summary

Procedure	Blk	TA	NSB	B ₀	Standards/Samples
Reconstitute and Mix	Mix all reagents gently				
Assay Buffer (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
DHEA-S AChE Tracer	--	--	50 µl	50 µl	50 µl
DHEA-S ELISA Monoclonal Antibody	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate for two hours at room temperature on an orbital shaker				
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Apply Ellman's Reagent	200 µl				
TA-Apply Tracer	--	5 µl	--	--	--
Develop	Seal plate and incubate for 60 minutes at room temperature on an orbital shaker, protected from light				
Read	Read absorbance at 405-420 nm				

Table 3. Assay summary

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

Warranty and Limitation of Remedy

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