Datasheet for ABIN2866592

Dehydroepiandrosterone Sulfate ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	Dehydroepiandrosterone Sulfate
Reactivity:	Human, Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Dehydroepiandrosterone sulfate Immunoassay kit uses a specifically generated an- tibody to measure Dehydroepiandrosterone sulfate (DHEA-S) in serum, plasma, urine, and saliva samples, and in fecal extracts.
Brand:	DetectX®
Sample Type:	Serum, Plasma, Urine, Saliva, Fecal, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Coated Clear 96 Well Plates Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG. 1 Or 5 each Dehydroepiandrosterone sulfate (DHEA-S) Standard Dehydroepiandrosterone sulfate at 1,200 ng/mL in a special stabilizing solution. 70 µL Or 350 µL DetectX® Dehydroepiandrosterone sulfate (DHEA-S) Antibody A sheep polyclonal antibody specific for dehydroepiandrosterone sulfate 3 mL Or 13 mL DetectX® Dehydroepiandrosterone sulfate (DHEA-S) Conjugate A dehydroepiandrosterone sulfate-peroxidase conjugate in a special stabilizing solution. 3 mL Or 13 mL

Product Details

	Assay Buffer Concentrate A 5X concentrate that must be diluted with deionized or distilled water. 28 mL Or 55 mL Wash Buffer Concentrate A 20X concentrate that must be diluted with deionized or distilled water. 30 mL Or 125 mL TMB Substrate Kit 11 mL Or 55 mL Stop Solution A 1M solution of hydrochloric acid. CAUSTIC. 5 mL Or 25 mL Plate Sealer 1 Or 5 each
Material not included:	Distilled or deionized water. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm. Ethanol or methanol for extraction of dried fecal samples. Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Target Details

Target:	Dehydroepiandrosterone Sulfate
Alternative Name:	DHEA-S (Dehydroepiandrosterone Sulfate Products)
Background:	Dehydroepiandrosterone sulfate, C19H28O5S, (5-androsten-3ß, 16a-diol-17-one sulfate, DHEA-S) is the major C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. It is produced by the addition of a sulfate group to dehydroepiandrosterone (DHEA), catalyzed by the sulfotransferase enzymes, SULT1A1 and SULT1E1, which also produce estrone sulfate from estrone. DHEA sulfate can also be back-converted to DHEA through the action of steroid sulfatase. Due to the the 17-ketone group rather than hydroxyl group, DHEA-S has relatively low androgenic activity (1). However the bioactivity of DHEA-S may be high due to its high serum concentrations at 100-1,000-fold higher than testosterone or DHEA and its weak affinity for sex-hormone binding globulin (2). Dehydroepiandrosterone sulfate The physiological role of DHEA-S is not well defined with serum levels being high in the fetus and neonates, low during childhood and increased during puberty (3, 4). DHEA-S levels decline during the third decade of life (5). DHEA-S, unlike DHEA and other steroids, does not show a significant diurnal or day-to-day variation. DHEA-S levels are not increased due to ACTH administration and do not change significantly during the normal menstrual cycle (2, 4). DHEA-S has a lower metabolic clearance rate than DHEA (6). Since DHEA-S is primarily produced by the adrenal glands, it is useful as a marker for adrenal function. Adrenal tumors, cancers, and hyperplasia can lead to the overproduction of DHEA-S.

Target Details

	While elevated levels may not be noticed in adult men, they can lead to amenorrhea and visible symptoms of virilization. These changes vary in severity and may include: a deeper voice, hirsutism, male pattern baldness, muscularity, acne and enlargement of the Adam's apple. Women with polycystic ovary syndrome tend to have elevated levels of DHEA-S. Excess levels of DHEA-S in children can cause precocious puberty in boys, and ambiguous external genitalia, excess body hair, and abnormal menstrual periods in girls

Application Details

Application Notes:	This assay has been validated for serum, plasma, saliva, urine, dried fecal extracts and for culture media samples. Samples containing visible particulate should be centrifuged prior to using. Dehydroepiandrosterone sulfate can be assayed in solid sample types by using one of the extraction protocols DHEA-S is identical across all species and we expect this kit to measure DHEA-S from all sources. The end user should evaluate recoveries of DHEA-S in other sample matrices being tested.
Plate:	Pre-coated
Protocol:	The kit will also quantitatively measure DHEA-S present in tissue culture media samples. Please read the complete kit insert before performing this assay. A DHEA- S standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture sheep antibodies. A DHEA-S-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to DHEA-S to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound DHEA-S-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the De- hydroepiandrosterone sulfate in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to

	accurately determine DHEA-S concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Assay Buffer Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deion- ized water. Once diluted this is stable at 4 °C for 3 months. Wash Buffer Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months. Standard Preparation Label test tubes #1 through #5. Pipet 380 µL of Assay Buffer into tube #1 and 160 µL into tubes #2 to #5. The Dehydroepiandrosterone sulfate (DHEA-S) stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 20 µL of the DHEA-S stock solution to tube #1 and vortex completely. Take 40 µL of the DHEA-S solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #5. The concentration of DHEA-S in tubes 1 through 5 will be 60,000, 12,000, 2,400, 480 and 96 pg/mL. Use all Standards within 2 hours of preparation.
Assay Procedure:	1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4ºC. 2. Pipet 50 µL of samples or standards into wells in the plate. 3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells. 4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL). 5. Add 25 µL of the DetectX® Dehydroepiandrosterone sulfate Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Dehydroepiandrosterone sulfate Antibody to each well, except the NSB wells, using a repeater pipet. 7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 20 % lower. 8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels. 9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

Application Details

	10. Incubate the plate at room temperature for 30 minutes without shaking. 11. Add 50 µL of the Stop Solution to each well, using a repeater pipet. 12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. 13. Use the plate reader's built-in 4PLC software capabilities to calculate Dehydroepiandrosterone sulfate concentration for each sample.
Calculation of Results:	Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values. Or, use the MyAssays™ online tool typical data Sample Mean OD Net OD % B/B0 Dehydroepiandrosterone sulfate (DHEA-S) Conc. (pg/mL) NSB 0.065 0 - - Standard 1 0.141 0.076 10.9 60,000 Standard 2 0.266 0.201 28.7 12,000 Standard 3 0.463 0.398 56.9 2,400 Standard 4 0.641 0.576 82.3 480 Standard 5 0.725 0.660 94.3 96 B0 0.765 0.700 100 0 Sample 1 0.308 0.243 34.7 8,208 Sample 2 0.413 0.348 49.6 3,580 Always run your own standard curve for calculation of results. Do not use this data. Conversion Factor: 100 pg/mL of Dehydroepiandrosterone sulfate is equivalent to 256.1 pM.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had labo- ratory safety instruction. The complete insert should be read and understood before attempting to use the product. The antibody coated plate must be stored desiccated. The silica gel pack included in the foil zi- ploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly. This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers' Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared. The Stop Solution is acid.

Handling

	The solution should not come in contact with skin or eyes. Take appro- priate precautions when handling this reagent.
Storage:	4 °C,RT
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Images

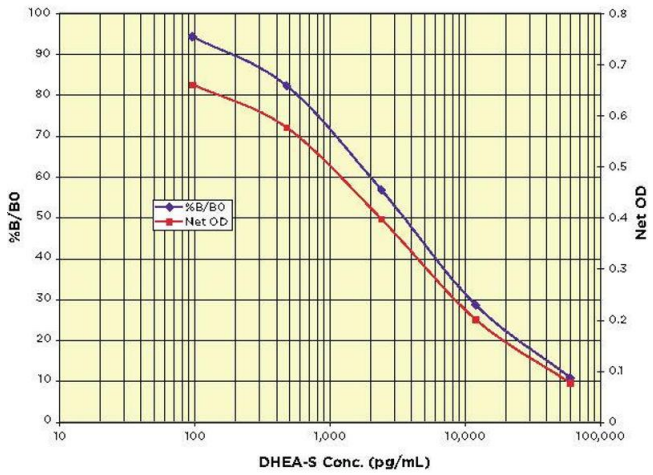

Image 1.