FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DHEAS ELISA Kit instruction

Intended use

This DHEAS ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures.The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of DHEAS in the sample, this DHEAS ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus DHEAS concentration. The concentration of DHEAS in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Sample collection and storages

1. Can't detect the samples which contain NaN3, because NaN3 inhibits HRP activity of the horseradish peroxidase.
2. Extract as soon as possible after Specimen collection, Extracted according to the relevant literature.

Cell culture supernates and plant exact fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw.

Materials required but not supplied

1. Standard microplate reader(450nm)
2. Precision pipettes and Disposable pipette tips.
3. 37 ℃ incubator

Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at

2-8°C in their pouch with the desiccant provided.

3. Mix all reagents before using.

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C)

Materials supplied

Name	96 determinations	48 determinations
Microelisa stripplate	8*12strips	8*6strips
Standard	0.3ml*6tubes	0.3ml*6tubes
Sample Diluent	6.0ml	3.0ml
HRP-Conjugate reagent	6.0ml	3.0ml
20X Wash solution	25ml	15ml
Chromogen Solution A	6.0ml	3.0ml
Chromogen Solution B	6.0ml	3.0ml
Stop Solution	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Note: Standard (S0 → S5) concentration was followed by:0、31.25、62.5、125、250、500 ng/mL

Reagent preparation

20×wash solution:Dilute with Distilled or deionized water 1:20.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.

2. Add standard: Set Standard wells, testing sample wells. Add standard 50µl to standard well.

3. Add Sample: Add testing sample 10µl then add Sample Diluent 40µl to testing sample well;

4. Add 50µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher.

Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**

7. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not

appear uniform, gently tap the plate to ensure thorough mixing.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

Calculation of results

1. Calculate the mean absorbance value A450 for each set of reference standards and samples.

2. Divide the average A450 value for each standard, control and test sample by the average A450 of standard0 and multiply by 100 to obtain %B/B0 for each sample.

3. Prepare a standard curve by plotting the average absorbance of each standard versus the corresponding concentrations of the standards on linear-log graph paper or the %B/B0 value for each standard versus the corresponding concentration of the standard on linear-log or logit-log graph paper. logit=ln(B/ B0)/(1－B/ B0)

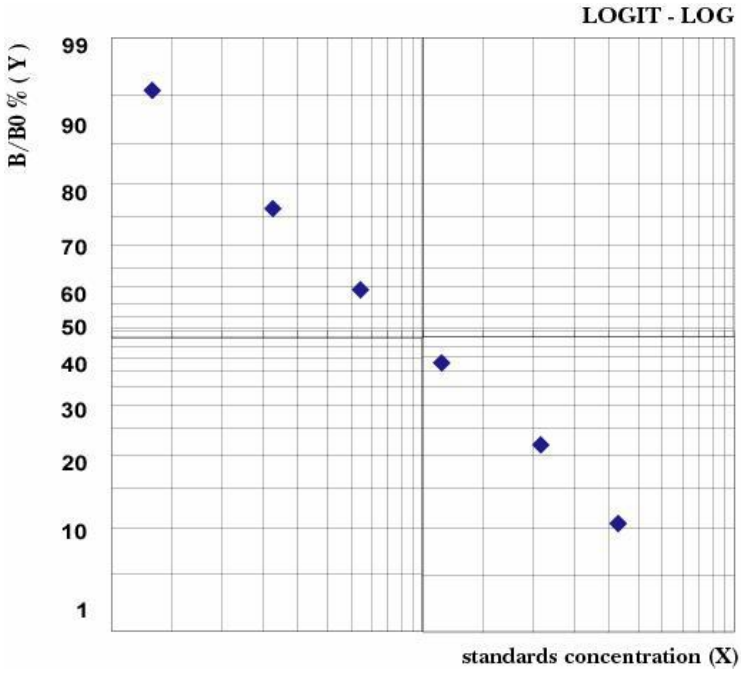
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

5. The standard density is a X, the B/ B0 is a Y, sitting to mark the paper in the logit- log up draw a standard curve. According to the B/ B0 that need to be measured the sample can from sit to mark the density value that the paper looks up the sample up.

6. The sensitivity by this assay is 1ng/mL

7. 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.

8. Standard curve match



Storage： 2-8℃.
Validity： six months.

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