Rat Dehydroepiandrosterone (DHEA) ELISA Kit

Product Code: 248663

Product Introduction

This kit is used to determine dehydroepiandrosterone (DHEA) content in samples.

This kit uses the double-antibody sandwich method to determine rat dehydroepiandrosterone (DHEA) levels. Purified rat dehydroepiandrosterone (DHEA) antibody is coated onto a microplate to prepare a solid-phase antibody. The wells are sequentially incubated with dehydroepiandrosterone (DHEA), and then with HRP-labeled dehydroepiandrosterone (DHEA) antibody to form an antibody-antigen-enzyme-labeled antibody complex.

After thorough washing, add substrate TMB for color development. TMB is converted to blue under the catalysis of HRP and turns yellow after the addition of acid stop solution. The color intensity is positively correlated with the dehydroepiandrosterone (DHEA) content in the sample. Measure absorbance (OD value) at 450 nm with a microplate reader, and calculate the rat dehydroepiandrosterone (DHEA) concentration in the sample from the standard curve.

Package Contents

Kit size: 48T

Code	Item	Specification
248663.1	20× Concentrated Wash Solution	20 mL × 1 bottle
248663.2	Enzyme-Labeled Reagent	3 mL × 1 bottle
248663.3	Sample Diluent	3 mL × 1 bottle
248663.4	Chromogenic Reagent A Solution	3 mL × 1 bottle
248663.5	Chromogenic Reagent B Solution	3 mL × 1 bottle
248663.6	Stop Solution	3 mL × 1 bottle
248663.7	Standard (96 nmol/L)	0.5 mL × 1 bottle
248663.8	Standard Diluent	1.5 mL × 1 bottle
248663.9	Coated ELISA Plate	12 wells × 4 strips
248663.10	Plate Sealing Film	2 sheets
248663.11	Sealed Bag	1 piece
248663.m	Manual	1 copy

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Main Toxicity
20× Concentrated Wash Solution	—	—
Enzyme-Labeled Reagent	—	—
Coated ELISA Plate	—	—
Sample Diluent	—	—
Color Reagent A Solution	—	—
Color Reagent B Solution	—	—
Stop Solution	—	—
Standard (96 nmol/L)	—	—
Standard Diluent	—	—
Sealing Film	—	—
Sealed Bag	—	—

Transportation and Storage

Transport: Transport with ice packs.

Storage: Store at 2-8°C. Shelf life: 180 days.

Instructions for Use

1. Procedure

1.1 Standard Dilution

This kit provides one undiluted standard. Perform serial dilution in small test tubes as follows:

Standard	Preparation
48 nmol/L Standard No. 5	Add 150 µL of the undiluted standard to 150 µL of standard diluent.
24 nmol/L Standard No. 4	Add 150 µL of Standard No. 5 to 150 µL of standard diluent.
12 nmol/L Standard No. 3	Add 150 µL of Standard No. 4 to 150 µL of standard diluent.
6 nmol/L Standard No. 2	Add 150 µL of Standard No. 3 to 150 µL of standard diluent.
3 nmol/L Standard No. 1	Add 150 µL of Standard No. 2 to 150 µL of standard diluent.

1.2 Assay Steps

1. Set up blank wells, standard wells, and sample wells. Do not add sample or enzyme conjugate reagent to the blank wells; all other steps are the same.
2. Add 50 µL of standard to each standard well.
3. Add 40 µL of sample diluent to each sample well, then add 10 µL of sample. The final sample dilution is 5-fold. Add liquid to the bottom of the well, avoid touching the wall, and shake gently to mix.
4. Seal the plate with sealing film and incubate at 37°C for 30 minutes.
5. Dilute the 20× concentrated wash solution 20-fold with distilled water before use.
6. Carefully remove the sealing film, discard the liquid, shake dry, and fill each well with wash solution. Let stand for 30 seconds, then discard. Repeat 5 times and pat dry.
7. Add 50 µL of enzyme conjugate reagent to each well except the blank well.
8. Seal and incubate again at 37°C for 30 minutes.
9. Wash again as described above.
10. Add 50 µL of chromogenic reagent A, then 50 µL of chromogenic reagent B. Shake gently to mix, then develop color at 37°C for 10 minutes protected from light.
11. Add 50 µL of stop solution to each well. The blue color immediately turns yellow.
12. Zero the instrument with the blank well and read the absorbance of each well at 450 nm. Complete the measurement within 15 minutes after adding the stop solution.

Calculation of Results

Use the concentration of the standards as the x-axis and the OD values as the y-axis to draw a standard curve on graph paper. Determine the corresponding sample concentration from the standard curve according to the sample OD value, then multiply by the dilution factor.

Alternatively, use the standard concentrations and OD values to calculate the linear regression equation of the standard curve. Substitute the sample OD value into the equation, then multiply by the dilution factor to obtain the actual sample concentration.

Assay Procedure Overview

1. Prepare reagents, samples, and standards.
2. Add the prepared samples and standards, then incubate at 37°C for 30 minutes.
3. Wash the plate 5 times, add enzyme conjugate reagent, and incubate at 37°C for 30 minutes.
4. Wash the plate 5 times, add chromogenic reagents A and B, and develop color at 37°C for 10 minutes.
5. Add stop solution.
6. Read the OD value within 15 minutes.

7. Calculate the results.

Precautions

1. After removal from refrigerated storage, equilibrate the kit to room temperature for 1 hour before use. If the coated plate is not fully used after opening, store the remaining strips in a sealed bag.
2. Crystals may precipitate from the concentrated wash solution. During dilution, warming in a water bath may help dissolution and does not affect washing performance.
3. Use a pipette for each sample addition step and check pipette accuracy frequently to avoid assay errors. It is best to keep the time for adding samples within 5 minutes. If there are many specimens, use a multichannel pipette.
4. Prepare a standard curve for each assay, and it is best to run duplicate wells. If the analyte content in a specimen is too high and the sample OD value is greater than that of the first standard well, dilute the sample with sample diluent by a factor of n before measurement. In the final calculation, multiply by the total dilution factor (×n×5).
5. Plate sealers are for one-time use only to avoid cross-contamination.
6. Store the substrate protected from light.
7. Strictly follow these instructions. Assay results must be based on the microplate reader readings.
8. All samples, wash solution, and various wastes should be treated as infectious materials.
9. Do not mix components from different batch numbers.
10. Sample requirements: Extract samples as soon as possible after collection and perform extraction according to relevant literature. After extraction, carry out the experiment as soon as possible. If testing cannot be performed immediately, samples may be stored at -20°C. Avoid repeated freeze-thaw cycles. Samples containing NaN3 cannot be tested because NaN3 inhibits horseradish peroxidase (HRP) activity.
11. Detection range of this kit: 2 nmol/L to 48 nmol/L.

> Important: Blank wells do not receive sample or enzyme conjugate reagent. Read the plate at 450 nm within 15 minutes after adding stop solution.

Visual Reference