Human Corticosterone ELISA Kit

Enzyme Immunoassay for the quantification of human Corticosterone in serum, and plasma

Catalog number: ARG80635

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Corticosterone is a glucocorticoid secreted by the cortex of the adrenal gland. Corticosterone is produced in response to the stimulation of the adrenal cortex by adrenocorticotropic hormone (ACTH) and is the precursor of aldosterone. Corticosterone is a major indicator of stress since stress increases the production of corticosteroids. Studies involving corticosterone and levels of stress include impairment of long term memory retrieval, chronic corticosterone elevation due to dietary restrictions and in response to burn injuries. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific Corticosterone antibody has been pre-coated onto a microtiter plate. Standards or samples and a fixed amount of enzyme-labeled antigen are pipetted into the wells and compete for the binding sites of the antibodies coated onto the wells. After incubation, the wells are washed to stop the competition reaction. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Corticosterone exist in the samples or standards. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm.The concentration of Corticosterone in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C.
Standards 0-6 (Concentration indicated on vial label)	7 vials/1 ml	4°C, ready for use
Control High	1 vial/ 1 ml	4°C, ready for use
Control Low	1 vial/ 1 ml	4°C, ready for use
250X Enzyme Conjugate antigen	1 vial	4°C, ready for use
Conjugate Diluent	25 ml	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	25 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.

- Briefly spin down all vials before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Enzyme Conjugate antibody: Dilute 250X Enzyme Conjugate antibody into Conjugate Diluent to yield 1X Enzyme Conjugate antibody.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 20 µl of standards, controls and samples in duplicate into wells.
3. Add 200 µl of Enzyme Conjugate antigen to each well. Cover wells and incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
6. Add 50 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

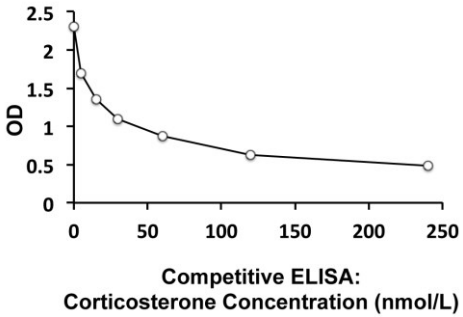
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Corticosterone ranged from 1.63-240 nmol/L. The mean MDD was <1.63 nmol/L.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.1% and inter-assay precision was 6.01%.

Specificity

No cross-reactivity has been found with the following factors:

Progesterone, Deoxycorticosterone, 11-Dehydrocorticosterone, Cortisol, Pregnolone, Other steroids

Recovery

95.2-105.7%

Linearity

95.0-103.9%