Cortisol ELISA Kit

Enzyme Immunoassay for the quantitative determination of Cortisol in dried fecal samples, serum, plasma (EDTA and heparin), tissue culture media and urine.

Catalog number: ARG81392

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Cortisol is a steroid hormone, in the glucocorticoid class of hormones. When used as a medication, it is known as hydrocortisone.

It is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland. It is released in response to stress and low blood-glucose concentration. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system, and to aid in the metabolism of fat, protein, and carbohydrates. It also decreases bone formation.

Cortisol is often referred to as the "stress hormone" as it is involved in the response to stress and it affects blood pressure, blood sugar levels, and other actions of stress adaptation. Immunologically, cortisol functions as an important anti-inflammatory and plays a role in hypersensitivity, immunosuppression, and disease resistance. In animals, cortisol is often used as an indicator of stress and can be measured in blood, saliva, urine, hair, and faeces [provide by Wikipedia]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. This Cortisol kit measures total Cortisol in dried fecal samples, serum, plasma (EDTA and heparin), tissue culture media and urine.

A specific anti-mouse-IgG antibody has been pre-coated onto a microtiter plate. Cortisol containing samples or standards and a Cortisol-HRP conjugate are given into the wells of the microtiter plate. Then a mouse monoclonal antibody to Cortisol is added into the wells. Cortisol in samples or standards and a fixed

amount of Cortisol-HRP conjugate are competing for a limited amount of Cortisol mouse monoclonal antibody. The Cortisol-antibody complex binds to the goat polyclonal anti-mouse IgG antibody that has been previously bound to the well.

After incubation, the wells are washed with wash buffer to remove unbound material. A substrate solution is then added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Cortisol is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Goat anti mouse IgG-coated microplate	96 wells	4°C
Standard (32000 pg/ml)	125 µl	-20°C
Cortisol monoclonal antibody	3 ml (ready to use)	4°C
100X Cortisol-peroxidase Conjugate	50 µl	-20°C
Conjugate Diluent Buffer	5 ml (ready to use)	4°C
Assay Buffer	50 ml (ready to use)	4°C
Dissociation Reagent	1 ml (ready to use)	4°C
10X Wash Buffer	50 ml	4°C
TMB substrate	11 ml (ready to use)	4°C (Protect from light)
STOP solution	5 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water
- ACS Grade Ethanol or Ethyl Acetate (optional)
- Glass test tubes (optional)
- Centrifugal vacuum devices (i.e., a SpeedVac™) (optional)
- 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 Standard and Cortisol-peroxidase Conjugate at -20°C, store other component at 4°C.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°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.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil zip lock bag will keep the plate dry.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Cortisol is identical across all species and we expect this kit may measure cortisol from sources other than human. The end user should evaluate recoveries of cortisol in other samples being tested. This assay has been validated for saliva, urine, serum and EDTA and heparin plasma samples, tissue culture samples and dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit.

Dried Fecal Samples

1. Ensure that the sample is completely dry and powder the sample to improve extraction recovery.
2. Remove any large particles, such as grass, if possible.

   (Note: We suggest checking the efficiency of extraction by preparing a steroid solution of known concentration in the kit Assay Buffer (AB).

   Spike one aliquot of your sample with a volume of the steroid solution in AB (Control Spike) and one aliquot of sample with the same volume of AB (Control Sample). Extract samples and both Controls with Ethanol or Ethyl Acetate as described below.)
3. Weigh out ≥ 0.2 mg of dried fecal solid into a tube.
4. Add 1 mL of Ethanol (or Ethyl Acetate) for every 0.1 mg of solid. (0.1 mg fecal solid/mL)
5. Shake vigorously for at least 30 minutes.
6. Centrifuge samples at 5,000 rpm for 15 minutes. Reserve supernatant in a clean tube. This material is able to be stored at ≤ -20°C for at least a month

if properly sealed.

7. Transfer a measured volume of supernatant (Evaporation Vol.) into a clean tube and evaporate to dryness in a SpeedVac or under nitrogen. Keep dried extracted samples frozen <-20°C in a desiccator.
   (Note: If only a portion of the organic solvent is being evaporated, ensure final amounts of measured steroid per mg solid accounts for volume of solution evaporated.)
8. Dissolve extracted sample with 100µL ethanol, followed by at least 400µL Assay Buffer (Reconstitution Vol.).
9. Vortex well and allow to sit 5 minutes at room temperature. Vortex and let sit for 5 minutes at room temperature twice more to ensure complete steroid solubility. For immunoassays **ethanol content in the well should be below ≤ 5%**. Dilute the ethanol-Assay Buffer mixture ≥1:10 with Assay Buffer to let ethanol content in the well below ≤ 5%
10. Run reconstituted diluted samples in assay immediately.
11. Determine the extraction efficiency by comparing the concentration of the steroid measured in the extracted Control (Control Spike- Control Sample) with the concentration of steroid before extraction.

Serum and Plasma Samples:

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 or heparin 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.

Preparation

1. Allow the Dissociation Reagent (DR) to warm completely to Room Temperature before use.
2. Add 5 µL of DR into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the DR in the tube, vortex gently and incubate at room temperature for 5 minutes or longer.
3. Add 490 µL of supplied Assay Buffer in the tube to dilute the sample. This 1:100 dilution can be diluted further with Assay Buffer.
4. The normal reference range for human serum cortisol is 2-25 µg/dL (20-250 ng/mL). Final serum and plasma dilutions should be ≥ 1:100

NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

Urine Samples

Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay. Urinary cortisol normally ranges from 0.7-119 µg/gram of creatinine or approximately 100,000 to 1,000,000 pg/mL in 24 hour urine samples. Samples may need to be diluted substantially to read within the standard curve range.

Tissue Culture Media

For measuring cortisol in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further

in TCM. We have validated the assay using RPMI-1640. (Use all Samples within 2 hours of preparation, or stored at ≤ -20°C until assaying.)

Note:

1. *Samples containing sodium azide should not be used in the assay.*
2. *Do not use haemolytic, icteric or lipaemic specimens.*

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water) The diluted Wash buffer is stable for 3 months at 4°C.
- 1X Cortisol-peroxidase conjugate: Dilute 100X Cortisol-peroxidase conjugate into Conjugate Diluent Buffer to yield 1X Cortisol-peroxidase conjugate. Store any unused Cortisol-peroxidase conjugate at-20°C.
- Standards: The cortisol stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery. The Assay Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 3200 pg/ml, 1600 pg/ml, 800 pg/ml, 400 pg/ml, 200 pg/ml, 100 pg/ml

Dilute cortisol standard as according to the table below:

Standard	Cortisol Conc. (pg/ml)	µl of Assay Buffer	µl of standard
S6	3200	450	50 (32,000 pg/mL stock)
S5	1600	250	250 (S6)
S4	800	250	250 (S5)
S3	400	250	250 (S4)
S2	200	250	250 (S3)
S1	100	250	250 (S2)
S0	0	250	0

(Note: Use all Standards within 2 hour of preparation. S0 as maximum binding (B0) or Zero standard)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) for 30 min before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add **50 µl** of the **Standards (S0 – S6)** and **diluted samples** into the appropriate wells.
3. Add **75 µl** of **Assay Buffer** into the non-specific binding (NSB) wells.
4. Add **50 µl** of **Assay Buffer** to act as maximum binding wells (pg/mL)
5. Add **25 µL** of the **Cortisol Conjugate** to each well using a repeater pipet.
6. Add **25 µL** of the **Cortisol 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 RT for 1 hour.**

8. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes.** Wash by filling each well with **1× Wash Buffer (300 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
9. Add **100 µl** of **TMB substrate solution** into each well.
10. Incubate for **30 mins at RT** with shaking. Avoid exposure to light.
11. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
12. 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 log-log, semi-log or 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. The Net OD = Mean OD – $OD_{NSB.}$

5. 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.

6. arigo provides GainData®, an in-house development ELISA data calculator,

for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

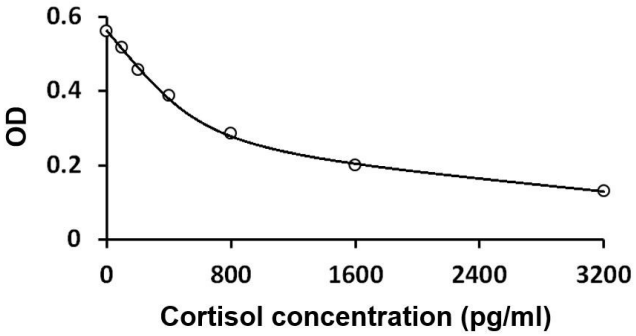
7. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

8. The sample concentrations obtained, calculated from the %B/B0 curve.

9. Conversion Factor: 100 pg/mL of cortisol is equivalent to 275.9 pM

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

Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the S0 and S6. The detection limit was determined at two (2) standard deviations from the S0 along the standard curve.

Sensitivity was determined as 30.3 pg/ml.

The Limit of Detection (LOD) for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the S0 and a low concentration of human sample. Limit of Detection was determined as 55.4 pg/mL

Intra and inter-assay precision

% CV of Intra assay precision: 8.8%

% CV of Inter assay precision: 8.13%

Specificity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Cortisol	100%
Prednisolone (1-Dehydrocortisol)	5.6%
Corticosterone	0.6%
11-Deoxycorticosterone	<0.1%
11-Hydroxyprogesterone	<0.1%
Progesterone	<0.1%
Estradiol	<0.1%
Danazol	<0.01%

Linearity

91-101.8%