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.

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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.

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 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 -20 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Goat anti IgG-coated microplate	96 wells	4°C
Standard (32000 pg/ml)	125 µl	4°C
Cortisol monoclonal antibody	3 ml	4°C
Cortisol-peroxidase conjugate	3 ml	4°C
5X Assay Buffer	28 ml	4°C
Dissociation Reagent	1 ml	4°C
20X Wash Buffer	30 ml	4°C
TMB substrate	11 ml (ready to use)	4°C (Protect from light)
STOP solution	5 ml (ready to use)	4°C
Plate Cover	1	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
- 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 kit at 4°C at all times.
- Briefly spin down the all vials before use.
- If crystals are observed in the 400X 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

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.
3. Weigh out ≥ 0.2 gm of dried fecal solid into a tube.
4. Add 1 mL of Ethanol (or Ethyl Acetate) for every 0.1 gm of solid. (0.1 gm 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 gm 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 twice more to ensure complete steroid solubility. For immunoassays ethanol content in the well should be below ≤ 5%. Dilute the ethanol-Assay Buffer mixture with Assay Buffer to let ethanol content in the well below ≤ 5%
10. Run reconstituted diluted samples in assay immediately.

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. We suggest pipeting 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. Dilute with 490 µL of supplied Assay Buffer. 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.

(Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying)

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water) The diluted Wash buffer is stable for 3 month at 4°C.
- 1X Assay Buffer: Dilute 5X Assay Buffer into distilled water to yield 1X Assay Buffer. (E.g. 10 ml of 5X Assay Buffer + 40 ml of distilled water) The diluted Assay Buffer is stable for 3 month at 4°C.
- Standards: The cortisol stock solution contains an organic solvent. Prerinse 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, 50 pg/ml,

Dilute cortisol standard as according to the table below:

Standard	Cortisol Conc. (pg/ml)	µl of Assay Buffer	µl of standard
S7	3200	450	50 (32,000 pg/mL stock)
S6	1600	250	250 (S7)
S5	800	250	250 (S6)
S4	400	250	250 (S5)
S3	200	250	250 (S4)
S2	100	250	250 (S3)
S1	50	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 and diluted samples into the appropriate wells.
3. Add 75 µl of Assay Buffer into the non-specific binding (NSB) wells.
4. Add 25 µL of the Cortisol Conjugate to each well using a repeater pipet.
5. Add 25 µL of the Cortisol Antibody to each well, except the NSB wells, using a repeater pipet
6. 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 1 hour.
7. 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
8. Add 100 µl of TMB substrate solution into each well. Incubate for 30 mins at RT without shaking. Avoid exposure to light.
9. Add 50 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at 450nm 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.

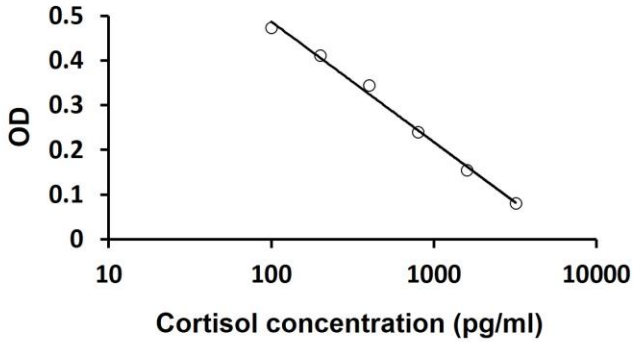
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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

7. 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

The minimum detectable dose (MDD) of Cortisol ranged from 100 - 3200 pg/ml. The mean MDD was 17.3pg/mL.

Intra and inter-assay precision

% CV of Intra assay precision: 5.6 - 14.7%

% CV of Inter assay precision: 6.3 - 10.9%

Specificity

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

Steroid	Cross Reactivity (%)
Cortisol	100%
Dexamethasone	18.8%
Prednisolone (1-Dehydrocortisol)	7.8%
Corticosterone	1.2%
Cortisone	1.2%
Progesterone	<0.1%
Estradiol	<0.1%
Cortisol 21-Glucuronide	<0.1%

Linearity

91-101%

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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