# Corticosterone EIA Kit

Item No. 500655

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## Materials Supplied

| Item Number | Item | 96 wells Quantity/Size | 480 wells Quantity/Size |
|---|---|---|---|
| 400656 | Corticosterone EIA Antiserum | 1 vial/100 dtn | 1 vial/500 dtn |
| 400655 | Corticosterone AChE Tracer | 1 vial/100 dtn | 1 vial/500 dtn |
| 400657 | Corticosterone EIA Standard | 1 vial | 1 vial |
| 400060 | EIA Buffer Concentrate (10X) | 2 vials/10 ml | 4 vials/10 ml |
| 400062 | Wash Buffer Concentrate (400X) | 1 vial/5 ml | 1 vial/12.5 ml |
| 400035 | Tween 20 | 1 vial/3 ml | 1 vial/3 ml |
| 400658/400659 | Rabbit Anti-Sheep IgG Coated Plate | 1 plate | 5 plates |
| 400012 | 96-Well Cover Sheet | 1 cover | 5 covers |
| 400050 | Ellman's Reagent | 3 vials/100 dtn | 6 vials/250 dtn |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

## Precautions

**Please read these instructions carefully before beginning this assay.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

**For research use only. Not for human or diagnostic use.**

## If You Have Problems

**Technical Service Contact Information**

| | |
|---|---|
| **Phone:** | 888-526-5351 (USA and Canada only) or 734-975-3888 |
| **Fax:** | 734-971-3641 |
| **Email:** | techserv@caymanchem.com |
| **Hours:** | M-F 8:00 AM to 5:30 PM EST |

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1.  A plate reader capable of measuring absorbance between 405-420 nm.

2.  Adjustable pipettes and a repeat pipettor.

3.  A source of 'UltraPure' water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*

4.  Materials used for **Sample Purification** (see page 13).

## Background

Corticosterone is a glucocorticoid produced by the adrenal cortex in response to ACTH (corticotropic hormone) and is the precursor to aldosterone. The production of glucorcorticoids is increased by stress; therefore, corticosterone can be used as a biomarker of stress.[1] Biologists often measure corticosterone in fecal material of the species they are studying.[1,2] This non-invasive sample collection procedure has the advantage of not itself causing stress and thereby increasing corticosterone levels, a common problem when samples for measurement of corticosterone are collected by more invasive means.[1] Plasma corticosterone levels have a circadian variation and corticosterone may be important in the regulation of the sleep-wake cycle.[2,3] The concentration of corticosterone in human plasma is approximately 0.9-3.9 ng/ml in females and 3.2-7.6 ng/ml in males.[4]

## About This Assay

Cayman's Corticosterone EIA Kit is a competitive assay that can be used for quantification of corticosterone in plasma and fecal samples. The EIA typically displays an $IC_{50}$ (50% $B/B_0$) of aproximately 150 pg/ml and a detection limit (80% $B/B_0$) of approximately 30 pg/ml.

## Description of ACE[TM] Competitive EIAs[5,6]

This assay is based on the competition between corticosterone and a corticosterone-acetylcholinesterase (AChE) conjugate (Corticosterone Tracer) for a limited number of corticosterone-specific sheep antiserum binding sites. Because the concentration of the Corticosterone Tracer is held constant while the concentration of corticosterone varies, the amount of Corticosterone Tracer that is able to bind to the sheep antiserum will be inversely proportional to the concentration of corticosterone in the well. This sheep antiserum-corticosterone (either free or tracer) complex binds to the rabbit polyclonal anti-sheep IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Corticosterone Tracer bound to the well, which is inversely proportional to the amount of free corticosterone present in the well during the incubation; or

$$\textbf{Absorbance} \propto [\textbf{Bound Corticosterone Tracer}] \propto 1/[\textbf{Corticosterone}]$$

A schematic of this process is shown in Figure 1, below.



Plates are pre-coated with rabbit anti-sheep IgG and blocked with a proprietary formulation of proteins.

1. Incubate with tracer, antiserum, and either standard or sample.

2. Wash to remove all unbound reagents.

3. Develop the well with Ellman's Reagent.

$\curlyvee$ = Rabbit Anti-Sheep IgG

━ = Blocking proteins

■○ = Acetylcholinesterase linked to Corticosterone (Tracer)

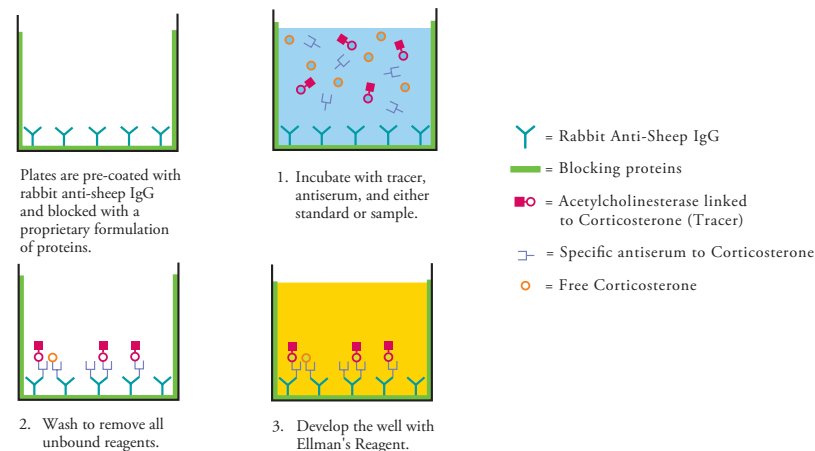= Specific antiserum to Corticosterone

○ = Free Corticosterone

**Figure 1. Schematic of the ACE[TM] EIA**

# Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *Electrophorus electricu*s, contains an avid acetylcholinesterase (AChE) capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover (64,000 s$^{-1}$) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 2, on page 9). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm ($\varepsilon = 13,600$).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows re-development of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts or preservatives. Since AChE is stable during the development step, it is unnecessary to use a 'stop' reagent, and the plate may be read whenever it is convenient.
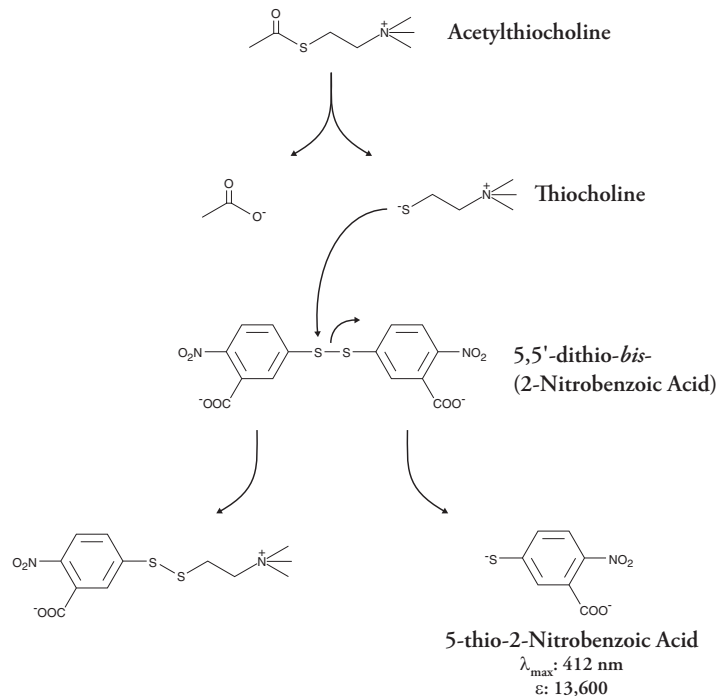


**Figure 2. Reaction catalyzed by acetylcholinesterase**

# Definition of Key Terms

**Blank:** background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

**Total Activity:** total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive tracer.

**NSB (Non-Specific Binding):** non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

**$B_0$ (Maximum Binding):** maximum amount of the tracer that the antibody can bind in the absence of free analyte.

**%B/$B_0$ (%Bound/Maximum Bound):** ratio of the absorbance of a particular sample or standard well to that of the maximum binding ($B_0$) well.

**Standard Curve:** a plot of the %B/$B_0$ values *versus* concentration of a series of wells containing various known amounts of analyte.

**Dtn:** determination, where one dtn is the amount of reagent used per well.

*NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).*

## Buffer Preparation

*Store all diluted buffers at 4°C; they will be stable for about two months*

1. **EIA Buffer Preparation**

   Dilute the contents of one vial of EIA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   **5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Item No. 400062):** Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Tween 20 (Item No. 400035).

   **OR**

   **12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Item No. 400062):** Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Tween 20 (Item No. 400035).

   Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Tween 20 (0.5 ml/liter of Wash Buffer).

   *NOTE: Tween 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

# Sample Preparation

This assay has been validated for plasma and fecal samples (see Figure 3, on page 16). Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

| General Precautions |
| --- |
| • All samples must be free of organic solvents prior to assay. |
| • Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. |
| • Samples of sheep origin may contain antibodies which interfere with the assay by binding to the rabbit anti-sheep IgG plate. We recommend that all sheep samples be purified prior to use in this assay. |

## Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate. To obtain plasma, spin samples at 1,000 x g for 15 minutes. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Process following the general **Sample Purification** methods beginning on page 13.

## Feces

Fecal samples should be frozen immediately after collection. In order to meet USDA requirements for receipt of international shipments of fecal material, it may be necessary to treat the samples with heat or chemicals to kill potential pathogens and prevent disease transmission. Millspaugh, *et al.,* have demonstrated that treatment of deer and elk fecal samples with 2% acetic acid does not alter the measurement of glucocorticoids in these samples.[3] Process following the general **Sample Purification** methods beginning on page 13.

# Sample Purification

This assay has been validated in plasma and feces. Other sample types should be checked for interference before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between approximately 30 and 800 pg/ml (*i.e.*, between ~20-80% $B/B_0$). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated corticosterone concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The **Plasma Extraction Protocol** on page 14, and the **Fecal Extraction Protocol** on page 17, may be used for this purpose.

## Determination of Recovery

Determination of percent recovery is recommended when any sample purification is performed. Detailed below are two methods that can be employed to monitor the recovery. If the **Hot Spike** method is used, 10,000 cpm of tritium-labeled corticosterone is added directly to the sample and 10% is removed for scintillation counting after purification. If the **Cold Spike** method is used, the sample must be split prior to purification and an appropriate amount of corticosterone added to one aliquot. The spiked sample is then assayed *via* EIA alongside the unspiked sample. Calculations for each method are found in the **Analysis** section on page 24.

## Purification Protocol

### Materials Needed

1. Tritium-labeled corticosterone to use as a **Hot Spike** or unlabeled Corticosterone Standard to use as a **Cold Spike** to allow determination of extraction efficiency.
2. Methylene chloride (if purifying plasma samples)
3. 90% Methanol (if purifying fecal samples)

### Plasma Sample Extraction

*NOTE: We do not recommend the use of plastic vials or caps for this procedure. The methylene chloride may extract interfering compounds from the plastic.*

| Hot Spike | Cold Spike |
|---|---|
| 1. Aliquot a known amount of each sample into a clean test tube. If your samples need to be concentrated, a larger volume should be used. <br><br> 2. Add 10,000 cpm of tritium-labeled corticosterone ([³H]-corticosterone). Use a high specific activity tracer to minimize the amount of radioactive cortico-sterone as the EIA will be able to detect the added corticosterone. Follow the procedure below for both spiked and unspiked samples. | 1. Aliquot a known amount of each sample into each of two tubes. Label the first tube 'sample #' and the second 'sample # + spike'. If your samples need to be concentrated, a larger volume should be used. <br><br> 2. Add a cold spike of corticosterone (5-10 ng/ml) to the 'sample + spike' tubes. Follow the procedure below for both spiked and unspiked samples. |

**Proceed to step 3 on next page**

3. Add methylene chloride (approximately four times the sample volume) to each tube. Vortex to mix thoroughly. Allow the layers to separate. Transfer the methylene chloride (lower) layer into a clean test tube using a transfer pipette. Repeat this step three times.

   *NOTE: If it is necessary to stop during this purification, samples may be stored in the methylene chloride solution at -20°C or -80°C.*

4. Evaporate the methylene chloride by heating to 30°C under a gentle stream of nitrogen.

5. Dissolve the extract in EIA Buffer. If you do not wish to concentrate your samples, use a volume of EIA Buffer equal to your sample volume. If you prefer to concentrate your samples, resuspend them in a volume smaller than the original volume. Use this sample for EIA analysis.

**Hot Spike (continued)**

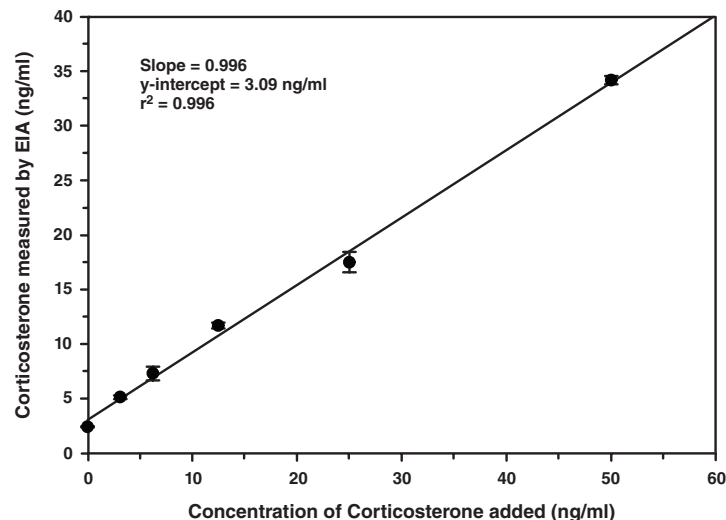6. Use 50 μl of the resuspended sample for scintillation counting.

**Figure 3. Recovery of corticosterone from human plasma**
Human plasma samples were spiked with corticosterone, purified as described in the **Sample Preparation** and **Sample Purification** sections and analyzed using the Corticosterone EIA Kit. The y-intercept corresponds to the amount of corticosterone in unspiked human plasma. Error bars represent standard deviations obtained from multiple dilutions of each sample.

## Fecal Sample Extraction

1. Put approximately 1 g of each sample into 20 ml plastic vials. *NOTE: This weight may need to be adjusted depending on the water content of the sample and the expected level of corticosterone in the sample.* Lyophilize.

2. Sift lyophilized samples through a stainless steel mesh to remove large particles. Mix each sample thoroughly to ensure homogeneity.

| Hot Spike | Cold Spike |
|---|---|
| 3. Place 0.2 g of each sample into clean test tubes. <br> 4. Add 2 ml of 90% methanol and 10,000 cpm of [$^3$H]-corticosterone to each sample. Follow the procedure below for both spiked and unspiked samples. | 3. Place 0.2 g of each sample into each of two tubes. Label the first tube 'sample #' and the second 'sample # + spike'. <br> 4. Add a cold spike of 5-10 ng of corticosterone to the 'sample + spike' tubes. Add 2 ml of 90% methanol to each tube. Follow the procedure below for both spiked and unspiked sample. |

### Proceed to step 5 below

5. Vortex at high speed for 30 minutes.

6. Centrifuge samples at 2,500 rpm for 20 minutes.

7. Transfer each supernatant to a clean test tube.

8. Evaporate under a gentle stream of nitrogen.

9. Dissolve the extract in 500 μl EIA Buffer. The sample is now ready for use in the EIA.

### Hot Spike (continued)

10. Use 50 μl of the reconstituted sample for scintillation counting.

## Preparation of Assay-Specific Reagents

### Corticosterone EIA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the Corticosterone EIA Standard (Item No. 400657) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 50 ng/ml. Store this solution at 4°C; it will be stable for at least six weeks.

*NOTE: If assaying culture medium samples that have not been diluted with EIA Buffer, culture medium should be used in place of EIA Buffer for dilution of the standard curve.*

To prepare the standard for use in EIA: obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 750 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (50 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.
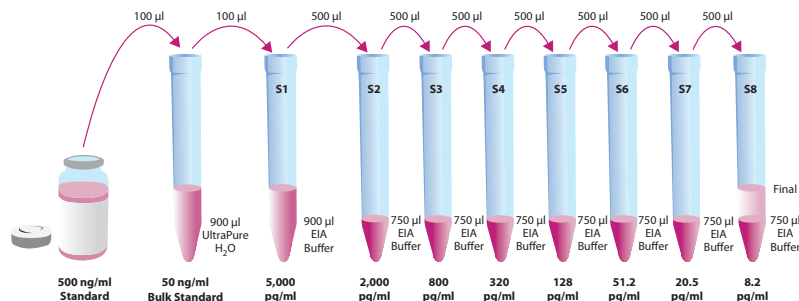


**Figure 4.  Preparation of the corticosterone standards**

### Corticosterone AChE Tracer

Reconstitute the Corticosterone AChE Tracer as follows:

> **100 dtn Corticosterone AChE Tracer (96-well kit; Item No. 400655):** Reconstitute with 6 ml EIA Buffer.

<div align="center">OR</div>

> **500 dtn Corticosterone AChE Tracer (480-well kit; Item No. 400655):** Reconstitute with 30 ml EIA Buffer.

Store the reconstituted Corticosterone AChE Tracer at 4°C *(do not freeze!)* and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

### Corticosterone EIA Antiserum

Reconstitute the Corticosterone EIA Antiserum as follows:

> **100 dtn Corticosterone EIA Antiserum (96-well kit; Item No. 400656):** Reconstitute with 6 ml EIA Buffer.

<div align="center">OR</div>

> **500 dtn Corticosterone EIA Antiserum (480-well kit; Item No. 400656):** Reconstitute with 30 ml EIA Buffer.

Store the reconstituted Corticosterone EIA Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

## Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells ($B_0$), and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 5, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 24, for more details). We suggest you record the contents of each well on the template sheet provided (see page 35).



Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
$B_0$ - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

**Figure 5.  Sample plate format**

## Performing the Assay

### Addition of the Reagents

1. **EIA Buffer**

   Add 100 μl EIA Buffer to Non-Specific Binding (NSB) wells. Add 50 μl EIA Buffer to Maximum Binding ($B_0$) wells. If culture medium was used to dilute the standard curve, substitute 50 μl of culture medium for EIA Buffer in the NSB and $B_0$ wells (*i.e.*, add 50 μl culture medium to NSB and $B_0$ wells and 50 μl EIA Buffer to NSB wells).

2. **Corticosterone EIA Standard**

   Add 50 μl from tube #8 to both of the lowest standard wells (S8). Add 50 μl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 50 μl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Corticosterone AChE Tracer**

   Add 50 μl to each well *except* the Total Activity (TA) and the Blank (Blk) wells.

5. **Corticosterone EIA Antiserum**

   Add 50 μl to each well *except* the Total Activity (TA), the Non-Specific Binding (NSB), and the Blank (Blk) wells.

| Well | EIA Buffer | Standard/Sample | Tracer | Antibody |
|---|---|---|---|---|
| Blk | - | - | - | - |
| TA | - | - | 5 µl (at devl. step) | - |
| NSB | 100 µl | - | 50 µl | - |
| $B_0$ | 50 µl | - | 50 µl | 50 µl |
| Std/Sample | - | 50 µl | 50 µl | 50 µl |

**Table 1. Pipetting summary**

## Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate for two hours at room temperature on an orbital shaker.

## Development of the Plate

1. Reconstitute Ellman's Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):

   **100 dtn vial Ellman's Reagent (96-well kit; Item No. 400050):** Reconstitute with 20 ml of UltraPure water.

   **OR**

   **250 dtn vial Ellman's Reagent (480-well kit; Item No. 400050):** Reconstitute with 50 ml of UltraPure water.

*NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays be run on different days.*

2. Empty the wells and rinse five times with Wash Buffer.

3. Add 200 µl of Ellman's Reagent to each well.

4. Add 5 µl of tracer to the Total Activity well.

5. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, $B_0$ wells ≥0.3 A.U. (blank subtracted)) in 60-90 minutes.

## Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.

2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. If Ellman's Reagent is present on the cover, use a pipette to transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.*

3. Read the plate at a wavelength between 405 and 420 nm (usually 412 nm). The absorbance may be checked periodically until the $B_0$ wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the $B_0$ wells are in the range of 0.3-1.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 2.0, wash the plate, add fresh Ellman's Reagent and let it develop again.

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/$B_0$ *versus* log concentration using a four-parameter logistic fit or as logit B/$B_0$ *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/eia) to obtain a free copy of this convenient data analysis tool.*

# Calculations

## Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

*NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.*

1. Average the absorbance readings from the NSB wells.

2. Average the absorbance readings from the $B_0$ wells.

3. Subtract the NSB average from the $B_0$ average. This is the corrected $B_0$ or corrected maximum binding.

4. Calculate the B/$B_0$ (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected $B_0$ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/$B_0$ for a logistic four-parameter fit, multiply these values by 100.)

*NOTE: The total activity (TA) values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected $B_0$ divided by the actual TA (10X measured absorbance) will give the % Bound. This value should closely approximate the % Bound that can be calculated from the **Sample Data** (see page 27). Erratic absorbance values and a low (or no) % Bound could indicate the presence of organic solvents in the buffer or other technical problems (see page 31 for **Troubleshooting**).*

## Plot the Standard Curve

Plot %B/$B_0$ for standards S1-S8 *versus* corticosterone concentration (usually in pg/ml) using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/$B_0$ in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/$B_0$) *versus* log concentrations and perform a linear regression fit.

## Determine the Sample Concentration

Calculate the B/$B_0$ (or %B/$B_0$) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well.* Samples with %B/$B_0$ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

<div style="text-align:center">**Hot Spike Method**</div>

$$\text{Recovery Factor} = \frac{10X \text{ cpm of sample}}{[^3H]\text{-Corticosterone added to sample}}$$

Corticosterone (pg) in purified sample =

$$\left[\frac{\text{Value from EIA (pg/ml)}}{\text{Recovery Factor}}\right] \text{ x reconstituted volume of sample - added } [^3H]\text{-Corticosterone (pg)}$$

**For liquid samples:**

$$\text{Corticosterone in sample (pg/ml)} = \frac{\text{Corticosterone (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

**For solid samples:**

$$\text{Corticosterone in sample (pg/g)} = \frac{\text{Corticosterone (pg) in purified sample}}{\text{weight of sample (g)}}$$

## Cold Spike Method

The original concentration of the sample and recovery factor can be determined by the following method:

V = EIA determined concentration of the unspiked sample (pg/ml)

S = concentration of the spike (pg/ml)

Y = EIA determined concentration of the spiked sample (pg/ml)

$$\text{Purification Recovery Factor} = \left[ \frac{Y - V}{S} \right]$$

$$\text{Corticosterone (pg) in purified sample} = \left[ \frac{V}{\text{Recovery Factor}} \right] \times \text{reconstituted volume of sample}$$

**For liquid samples:**

$$\text{Corticosterone in original sample (pg/ml)} = \frac{\text{Corticosterone (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

**For solid samples:**

$$\text{Corticosterone in original sample (pg/g)} = \frac{\text{Corticosterone (pg) in purified sample}}{\text{Weight of sample (g)}}$$
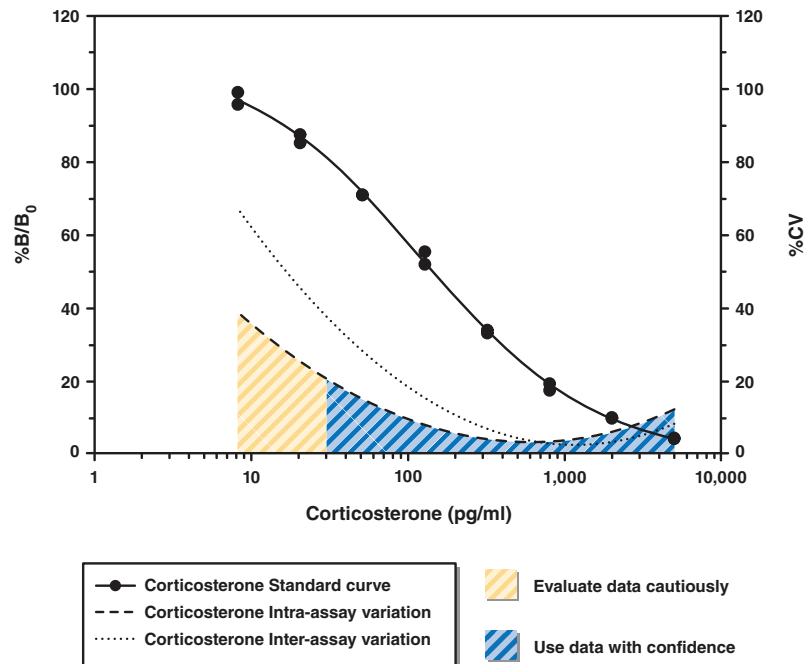
## Performance Characteristics

### Sample Data

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. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

|  | Raw Data | | Average | Corrected |
|---|---|---|---|---|
| **Total Activity** | 2.717 | 2.744 | 2.730 | |
| **NSB** | 0.001 | 0.006 | 0.003 | |
| **$B_0$** | 1.027 | 1.038 | | |
|  | 1.054 | 1.092 | 1.053 | 1.050 |

| Dose (pg/ml) | Raw Data | | Corrected | | %B/B$_0$ | |
|---|---|---|---|---|---|---|
| 5,000 | 0.048 | 0.051 | 0.045 | 0.048 | 4.3 | 4.6 |
| 2,000 | 0.110 | 0.108 | 0.107 | 0.105 | 10.2 | 10.0 |
| 800 | 0.208 | 0.188 | 0.205 | 0.185 | 19.5 | 17.6 |
| 320 | 0.353 | 0.361 | 0.350 | 0.358 | 33.3 | 34.0 |
| 128 | 0.587 | 0.551 | 0.584 | 0.548 | 55.5 | 52.1 |
| 51.2 | 0.751 | 0.751 | 0.748 | 0.748 | 71.0 | 71.0 |
| 20.5 | 0.901 | 0.924 | 0.898 | 0.921 | 85.3 | 87.5 |
| 8.2 | 1.046 | 1.011 | 1.043 | 1.008 | 99.1 | 95.7 |

**Table 2.  Typical results**

**Figure 6. Typical standard curve**

Corticosterone Standard curve
Corticosterone Intra-assay variation
Corticosterone Inter-assay variation

Evaluate data cautiously
Use data with confidence

50% $B/B_0$ - 150 pg/ml

Detection Limit (80% $B/B_0$) - 30 pg/ml

## Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 28 and in the table below.

| Dose (pg/ml) | %CV* Intra-assay variation | %CV* Inter-assay variation |
|---|---|---|
| 5,000 | 10.9 | 6.2 |
| 2,000 | 5.6 | 3.8 |
| 800 | 6.0 | 6.6 |
| 320 | 8.0 | 9.8 |
| 128 | 7.5 | 14.6 |
| 51.2 | 12.5 | 22.5 |
| 20.5 | 20.3 | † |
| 8.2 | † | † |

**Table 3. Intra- and inter-assay variation**
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

**Specificity:**

| Compound | Cross-reactivity |
|---|---|
| Corticosterone | 100% |
| 11-Dehydrocorticosterone | 11% |
| 11-Deoxycorticosterone | 7% |
| Progesterone | 0.31% |
| Cortisol | 0.17% |
| Aldosterone | 0.06% |
| Testosterone | 0.03% |
| Pregnenolone | 0.02% |
| 5$\alpha$-DHT | 0.01% |
| Androstenedione | <0.01% |
| Cortisone | <0.01% |
| DHEA | <0.01% |
| DHEA-S | <0.01% |

**Table 4.  Specificity of the Corticosterone EIA Antiserum**

# Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates | A. Trace organic contaminants in the water source<br>B. Poor pipetting/technique | A. Replace activated carbon filter or change source of UltraPure water |
| High NSB (>0.035) | A. Poor washing<br>B. Exposure of NSB wells to specific antibody | A. Re-wash plate and redevelop |
| Very low $B_0$ | A. Trace organic contaminants in the water source<br>B. Plate requires additional development time<br>C. Dilution error in preparing reagents | A. Replace activated carbon filter or change source of UltraPure water<br>B. Return plate to shaker and re-read later |
| Low sensitivity (shift in dose response curve) | Standard is degraded | Replace standard |
| Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference) | Interfering substances are present | Purify sample prior to analysis by EIA[7] |
| Only Total Activity (TA) wells develop | Trace organic contaminants in the water source | Replace activated carbon filter or change source of UltraPure water |

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# Related Products

Aldosterone EIA Kit - Monoclonal - Item No. 10004377
Cortisol EIA Kit - Item No. 500360
Cortisol Express EIA Kit - Item No. 500370
Estradiol EIA Kit - Item No. 582251
Estriol EIA Kit - Item No. 582281
Progesterone EIA Kit - Item No. 582601
Testosterone EIA Kit - Item No. 582701
UltraPure Water - Item No. 400000

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# NOTES