Testosterone EIA Kit

Item No. 582701

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Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
482702	Testosterone EIA Antiserum	1 vial/100 dtn	1 vial/500 dtn
482700	Testosterone AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
482704	Testosterone 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	Polysorbate 20	1 vial/3 ml	1 vial/3 ml
400004/400006	Mouse Anti-Rabbit 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
400040	EIA Tracer Dye	1 vial	1 vial
400042	EIA Antiserum Dye	1 vial	1 vial

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.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

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.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

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 Preparation** (see page 12).

INTRODUCTION

Background

Testosterone is the prototypic and predominant circulating androgenic steroid. It plays a major role in the growth and function of many reproductive and non-reproductive tissues and organs including muscle, liver, and brain, directing the development of the male phenotype during embryogenesis and at puberty. Testosterone is synthesized from pregnenolone by two main pathways, the Δ^4 and Δ^5 pathways. Both of these pathways involve the same three enzymes: the cytochrome P450 isoform CYP17, 3β-hydroxysteroid dehydrogenase/isomerase, and 17β-hydroxysteroid dehydrogenase. The human CYP17 favors the Δ^5 pathway, in which 17-hydroxypregnenolone is converted first to DHEA, then androstenedione, and finally testosterone. The Δ^4 pathway in which 17-hydroxyprogesterone is converted to androstedione is also important, but less favored in humans.[1] While testosterone is the androgen responsible for biological activity in tissues such as muscle, in other tissues including gonads and skin, it is converted to 5α-DHT by the action of 5α-reductase. 5α-DHT binds to the androgen receptor with higher affinity than testosterone itself and mediates androgenic activity in tissues where it is present. Aromatase (CYP19) is responsible for the conversion of testosterone to estradiol.[1] Total serum testosterone levels range from <1 ng/ml in women to 3-10 ng/ml in men, declining with age.[1]The majority of testosterone is glucuronidated prior to excretion in urine.[2,3]

About This Assay

Cayman's Testosterone EIA Kit is a competitive assay that can be used for quantification of testosterone in serum, plasma, tissue culture supernatants and other sample matrices. The assay does not recognize testosterone-17-glucuronide, the metabolite most prevalent in urine, and therefore is not recommended for use with urine samples. The EIA typically displays an IC_{50} (50% B/B_0) of approximately 32 pg/ml and a detection limit (80% B/B_0) of approximately 6 pg/ml.

Description of ACE™ Competitive EIAs

This assay is based on the competition between testosterone and a Testosterone-acetylcholinesterase (AChE) conjugate (Testosterone Tracer) for a limited amount of Testosterone Antiserum. Because the concentration of the Testosterone Tracer is held constant while the concentration of testosterone varies, the amount of Testosterone Tracer that is able to bind to the Testosterone Antiserum will be inversely proportional to the concentration of testosterone in the well. This antiserum-testosterone complex binds to mouse monoclonal anti-rabbit 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 Testosterone Tracer bound to the well, which is inversely proportional to the amount of free Testosterone present in the well during the incubation; or

Absorbance ∝ [Bound Testosterone Tracer] ∝ 1/[Testosterone]

A schematic of this process is shown in Figure 1.

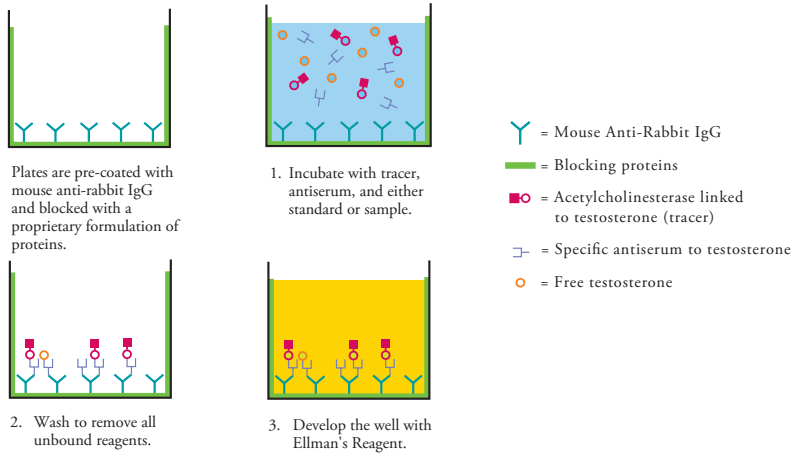


Figure 1. Schematic of the ACE™ EIA

Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *Electrophorus electricus*, 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 (ε = 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.

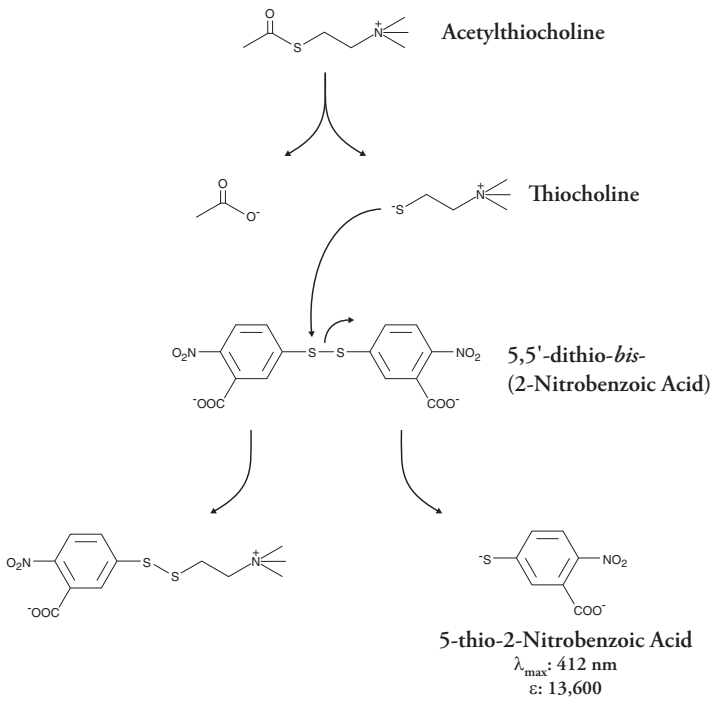


Figure 2. Reaction catalyzed by acetylcholinesterase

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.

PRE-ASSAY PREPARATION

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 Polysorbate 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 Polysorbate 20 (Item No. 400035).

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

NOTE: Polysorbate 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

In general, tissue culture supernatant samples may be diluted with EIA Buffer and added directly to well (see **General Precautions** section for more details). Plasma and serum, as well as other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants which can interfere in the assay. It is best to check 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 within the linear range of the assay (approximately 20-80% B/B_0). If the two different dilutions of the sample show good correlation (differ by 20% or less), purification is not required. If you do not see good correlation at the different dilutions, purification is advised. The Extraction Protocol, on page 13, is one such method.

General Precautions

- All samples must be free of organic solvents prior to assay.
- AEBSF (Pefabloc SC®) and PMSF inhibit acetylcholinesterase. Samples containing these protease inhibitors should not be used in this assay.
- Fetal bovine serum (FBS) may contain significant amounts of steroids that could interfere with this assay. For best results, we recommend using FBS that has been charcoal stripped or dialyzed to remove free hormone.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -20 or -80°C.
- Samples of rabbit origin may contain antibodies which interfere with the assay by binding to the mouse anti-rabbit IgG plate. We recommend that all rabbit samples be purified prior to use in this assay.

Tissue Culture Supernatants

Tissue culture supernatants may be diluted with EIA Buffer and added directly to the well.

Serum and Plasma

Serum and plasma contain a number of other steroids that may interfere with this assay. It is essential that these samples be extracted in order to obtain accurate results. Failure to extract samples may result in spurious data

Extraction Protocol

The following protocol is recommended for extraction of serum and plasma samples. Use of this procedure will allow the quantification of total testosterone. If desired, recovery may be traced by spiking samples with tritium-labeled testosterone ($[^3H]$-testosterone) and following the spiked-sample recovery calculations in the **Analysis** section, on page 22. Otherwise, omit step 2.

NOTE: We do not recommend the use of plastic vials, caps, or pipettes for this procedure. The ether may extract interfering compounds from the plastic. For best results, avoid the use of ether that has been stored in a container containing polypropylene, polyethylene, or polystyrene.

1. Aliquot a known amount of each sample into a clean tube (500 µl is recommended).
2. Add 10,000 cpm of tritium-labeled testosterone ($[^3H]$-testosterone). Use a high specific activity tracer to minimize the amount of radioactive testosterone as the EIA will be able to detect the added testosterone.
3. Add 5X the sample volume of diethyl ether and mix thoroughly with a vortexer. Allow the layers to separate. Using a pasteur pipette, transfer 90% of the ether (upper layer) into a clean test tube. Repeat this extraction three more times.*
4. Evaporate the combined ether extracts by heating to 30°C under a gentle stream of nitrogen.
5. Dissolve the extract in 0.5 ml of EIA Buffer. Use this sample for EIA analysis.

*If it is necessary to stop during this extraction, samples may be stored in the diethyl ether solution at -20°C or -80°C.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Testosterone 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 Testosterone EIA Standard (Item No. 482704) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 5 ng/ml.

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 500 µl EIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (5 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.

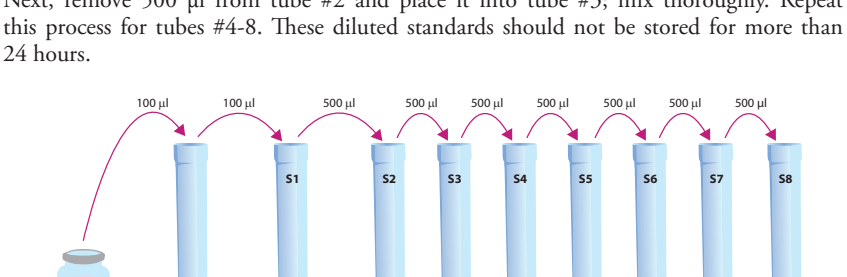


Figure 3. Preparation of the Testosterone standards

Testosterone AChE Tracer

Reconstitute the Testosterone AChE Tracer as follows:

100 dtn Testosterone AChE Tracer (96-well kit; Item No. 482700): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn Testosterone AChE Tracer (480-well kit; Item No. 482700): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted Testosterone 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.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer or add 300 µl of dye to 30 ml of tracer).

Testosterone EIA Antiserum

Reconstitute the Testosterone EIA Antiserum as follows:

100 dtn Testosterone EIA Antiserum (96-well kit; Item No. 482702): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn Testosterone EIA Antiserum (480-well kit; Item No. 482702): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted Testosterone 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.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum or add 300 µl of dye to 30 ml of antiserum).

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 4, 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 20, for more details). We suggest you record the contents of each well on the template sheet provided (see page 31).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

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

Figure 4. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

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. Testosterone 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. Testosterone AChE Tracer

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

5. Testosterone 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 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 wells.
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. 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 1.5, wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

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 23). 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 27 for* ***Troubleshooting****).*

Plot the Standard Curve

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

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

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

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.

Spiked-Sample Recovery Calculation

$$\text{Recovery Factor} = \frac{10 \times \text{cpm of sample}}{[^3\text{H}]\text{-Testosterone added to sample (cpm)}}$$

Testosterone (pg) in purified sample =

$$\left[\frac{\text{Value from EIA (pg/ml)}}{\text{Recovery Factor}}\right] \times 0.5 \text{ ml} - \text{added } [^3\text{H}]\text{-Testosterone (pg)}$$

Total Testosterone in sample (pg/ml) =

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

Performance Characteristics

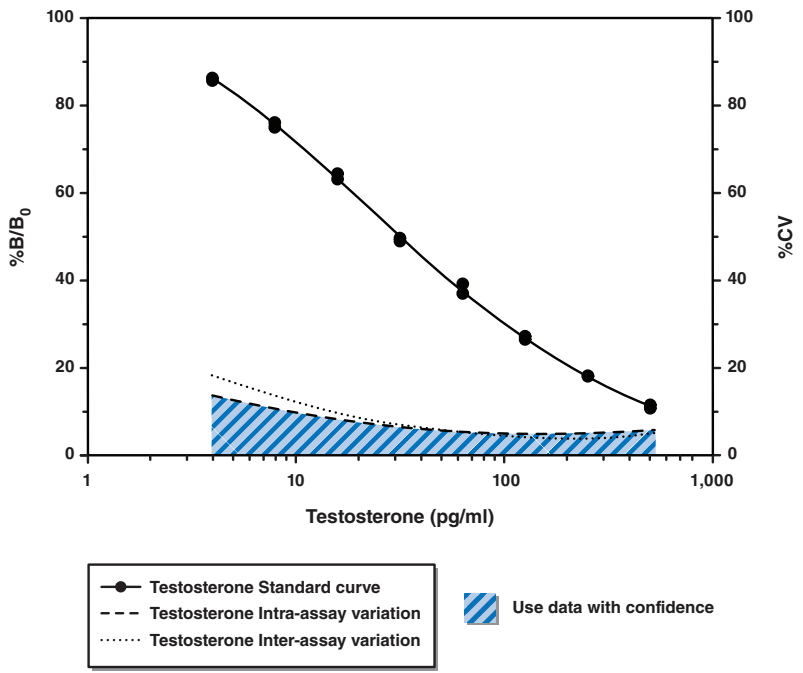
Sample Data

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	1.247	1.465	1.356	
NSB	0.000	0.000	0.000	
B_0	0.806	0.781		
	0.828	0.852	0.817	0.817

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
500	0.098	0.092	0.098	0.092	12.05	11.24
250	0.152	0.152	0.152	0.152	18.65	18.55
125	0.221	0.227	0.221	0.227	27.00	27.74
62.5	0.306	0.324	0.306	0.324	37.51	39.69
31.3	0.404	0.410	0.404	0.410	49.52	50.17
15.6	0.520	0.530	0.520	0.530	63.72	64.94
7.8	0.626	0.617	0.626	0.617	76.66	75.58
3.9	0.705	0.709	0.705	0.709	86.30	86.86

Table 2. Typical results



50% B/B_0 - 32 pg/ml
Detection Limit (80% B/B_0) - 6 pg/ml

Figure 5. Typical standard curve

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 24 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
500	4.4	7.7
250	4.6	2.8
125	6.1	4.7
62.5	6.0	7.2
31.3	6.6	7.5
15.6	9.9	8.5
7.8	14.0	10.7
3.9	19.1	14.2

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

Specificity:

Compound	Cross Reactivity
19-Nortestosterone	140%
Testosterone	100%
5α-dihydrotestosterone	27.4%
5β-dihydrotestosterone	18.9%
Methyl Testosterone	4.7%
Androstenedione	3.7%
11-keto Testosterone	2.2%
5-Androstenediol	0.51%
Epi-Testosterone	0.2%
Progesterone	0.14%
Testosterone Enanthate	0.11%
Androsterone	0.05%
Androsterone Sulfate	0.04%
Testosterone Sulfate	0.03%
DHEA Sulfate	0.02%
Estradiol	<0.01%
Testosterone Glucuronide	<0.01%

Table 3. Specificity of the Testosterone Antiserum

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
High NSB (>0.035)	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Rewash plate and redevelop
Very low B_0	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents	A. Replace activated carbon filter or change source of UltraPure water 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[4]
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

References

1. Kicman, A.T. Biochemical and physiological aspects of endogenous androgens. *Handb. Exp. Pharmacol.* **195**, 25-64 (2010).
2. Gomes, R.L., Meredith, W., Snape, C.E., *et al.* Analysis of conjugated steroid androgens: Deconjugation, derivatisation and associated issues. *J. Pharm. Biomed. Anal.* **49(5)**, 1133-1140 (2009).
3. Gower, D.B. Analysis of androgens and their derivatives, Chapter 6, *in* Steroid Analysis. Makin, H.L.J. and Gower, D.B., editors, 2nd, Springer, New York, 457-558 (2010).
4. Maxey, K.M., Maddipati, K.R. and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**,116-120 (1992).

Related Products

Aldosterone EIA Kit - Monoclonal - Item No. 10004377
Arginine Vasopressin EIA Kit - Item No. 583951
Corticosterone EIA Kit - Item No. 500655
Estradiol EIA Kit - Item No. 582251
Estriol EIA Kit - Item No. 582281
Follicle-Stimulating Hormone EIA Kit - Item No. 500710
Luteinizing Hormone EIA Kit - Item No. 500720
Oxytocin EIA Kit - Item No. 500440
Prolactin (human) EIA Kit - Item No. 500730
Progesterone EIA Kit - Item No. 582601
11-keto Testosterone EIA Kit - Item No. 582751
UltraPure Water - Item No. 400000

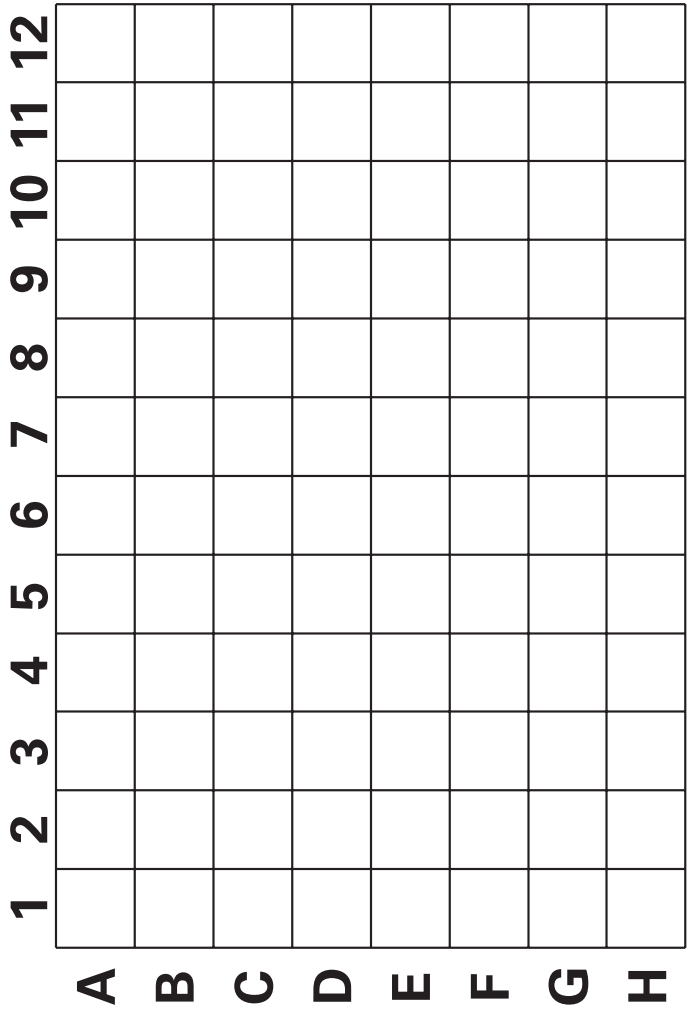
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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