Testosterone ELISA Kit

Item No. 582701

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Safey Data
4 Precautions
5 If You Have Problems
5 Storage and Stability
5 Materials Needed but Not Supplied

INTRODUCTION
6 Background
6 About This Assay
7 Principle of This Assay
8 Definition of Key Terms

PRE-ASSAY PREPARATION
10 Buffer Preparation
11 Sample Preparation
14 Sample Matrix Properties

ASSAY PROTOCOL
18 Preparation of Assay-Specific Reagents
21 Plate Set Up
22 Performing the Assay

ANALYSIS
26 Calculations
28 Performance Characteristics

RESOURCES
32 Troubleshooting
33 References
34 Plate Template
35 Notes
35 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
482702	Testosterone ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
482700	Testosterone AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
482704	Testosterone ELISA Standard	1 vial	1 vial
400060	ELISA 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 ea	5 ea
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	ELISA Tracer Dye	1 vial	1 vial
400042	ELISA 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) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

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 Testosterone ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

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

Email: techserv@caymanchem.com

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 repeating pipettor.
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 11).

INTRODUCTION

Background

Testosterone is the prototypic and predominant circulating androgenic steroid.[1,2] 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.[1] 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 (CYP) isoform CYP17, 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-HSD. The human CYP17 favors the Δ^5 pathway, in which 17-hydroxypregnenolone is converted first to dehydroepiandrosterone (DHEA), then androstenedione, and finally testosterone. The Δ^4 pathway in which 17-hydroxyprogesterone is converted to androstenedione is also important, but less favored in humans. 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. Total serum testosterone levels range from <1 ng/ml in women to 3-10 ng/ml in men, declining with age. The majority of testosterone is glucuronidated prior to excretion in urine.[2]

About This Assay

Cayman's Testosterone ELISA Kit is a competitive assay that can be used for quantification of testosterone in plasma, serum, saliva, tissue culture supernatants, animal feces, 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 assay has a range from 3.9-500 pg/ml, a sensitivity (80% B/B_0) of approximately 6 pg/ml, and a lower limit of detection (LLOD) of 3 pg/ml.

Principle of This Assay

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

$$\text{Absorbance} \propto [\text{Bound Testosterone Tracer}] \propto 1/[\text{Testosterone}]$$

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

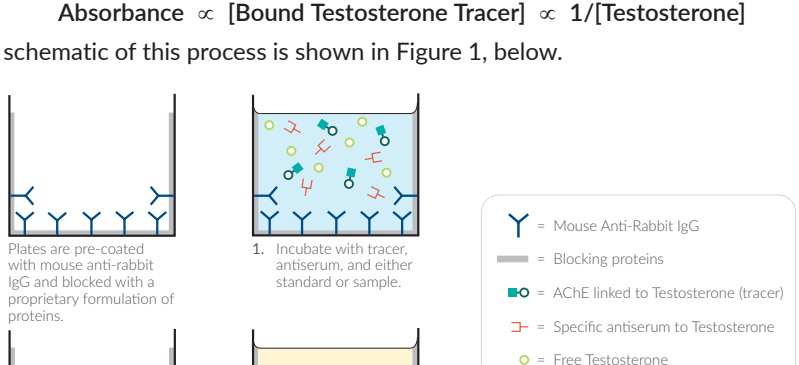


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (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. Do not forget to subtract the Blank absorbance values.

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): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% B/B}_0 \text{ value for the primary analyte}}{\text{50\% B/B}_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all ELISA 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 ELISA. 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. **ELISA Buffer (1X) Preparation**

   Dilute the contents of one vial of ELISA 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 (1X) 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 (1X) can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer (1X)).

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

Testing for Interference

This assay has been validated in human plasma, serum, saliva, cell culture supernatants, and animal feces. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on an 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 portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated testosterone concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. purification methods will be determined by the end user and tested for compatibility in the assay.

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°C 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

Some components of the tissue culture medium may interfere with ELISA. If a high concentration of the analyte is expected, tissue culture supernatants may be diluted with ELISA Buffer (1X) and added directly to the well. If testing samples at a low dilution (1:2-1:5), we recommend using a standard curve prepared in the ELISA Buffer (1X) containing the same concentration of the tissue culture medium as in your diluted samples. If testing undiluted samples, prepare standard curve in tissue culture medium. Use the corresponding medium or its solution in place of the buffer in NSB and B_0 wells.

Plasma, Serum, and Saliva

Plasma, serum, and saliva 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.

The following protocol is a suggestion only. You may choose a different protocol based on your own requirements, sample type and expertise.

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 (0.5 ml is recommended).
2. 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.*
3. Evaporate the combined ether extracts under a gentle stream of nitrogen.
4. Dissolve the extract in 0.5 ml of ELISA Buffer (1X). Use this sample for ELISA 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.

Feces

1. Lyophilize feces to remove excess water.
2. Homogenize lyophilized feces into a fine powder. The use of personal protection equipment, especially respirators is advised while working with lyophilized feces.
3. Place 25 mg of fine lyophilized feces into a centrifuge tube, add 200 µl of ultrapure water, and vortex thoroughly.
4. Add 800 µl of methanol, vortex and agitate on multi-tube vortexer for one hour at room temperature.
5. Centrifuge at 2,000 x g for 5 minutes.
6. Transfer supernatant into a clean glass test tube.
7. Resuspend the pellet in 200 µl of ultrapure water, vortex, add 800 µl of methanol, vortex and agitate on multi-tube vortexer for one hour at room temperature.
8. Centrifuge at 2,000 x g for 5 minutes. Transfer supernatant into the same glass test tube from step 6.
9. Evaporate supernatant under a gentle stream of nitrogen by heating sample to 37°C.
10. Reconstitute in 400 µl of ELISA Buffer (1X) and test in the assay.

Sample Matrix Properties

Linearity

To assess dilutional linearity, human plasma and serum samples were spiked with 6,000 or 1,250 pg/ml testosterone and purified as described in the sample preparation section. All purified samples were serially diluted with ELISA Buffer (1X), and evaluated for linearity using the Testosterone ELISA Kit. The results are shown in Table 1, on page 15.

Dilution Factor	Testosterone (pg/ml)	Dilutional Linearity (%)
Human Plasma, spike 6,000 pg/ml		
20	6,735	100
40	7,083	105
80	7,000	104
Human Plasma, spike 1,250 pg/ml		
10	1,592	100
20	1,617	102
40	1,633	103
Human Serum, spike 6,000 pg/ml		
20	8,287	100
40	8,396	101
80	8,650	104
Human Serum, spike 1,250 pg/ml		
10	1,511	100
20	1,404	93
40	1,420	94

Table 1. Dilutional linearity of human plasma and serum samples
NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

Spike and Recovery

Human plasma and serum were spiked with different amounts of testosterone and purified as described in the Sample Preparation section. All purified samples were serially diluted with ELISA Buffer (1X) and analyzed using the Testosterone ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

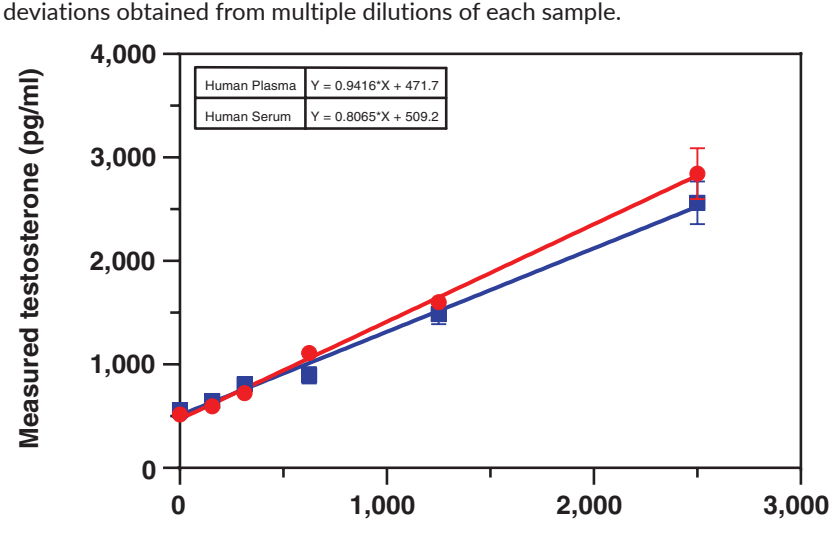


Figure 2. Spike and recovery of testosterone in human plasma and serum

Parallelism

To assess parallelism, human plasma and serum samples were purified as described in the Sample Preparation section, serially diluted, and tested using the Testosterone ELISA Kit. Measured concentrations were plotted as a function of sample dilution. The results are shown below.

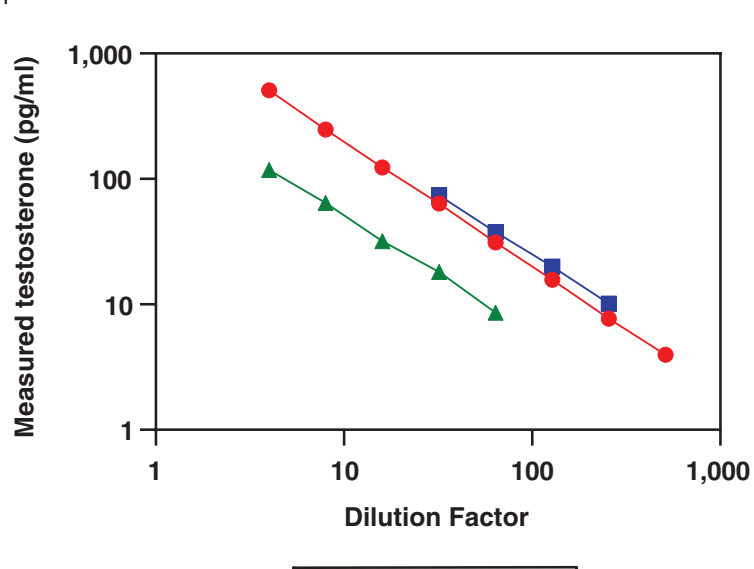


Figure 3. Parallelism of human plasma and serum in the Testosterone ELISA

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Testosterone ELISA 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 ELISA 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 ELISA Buffer (1X), culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl ELISA Buffer (1X) to tube #1 and 500 µl ELISA Buffer (1X) 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.

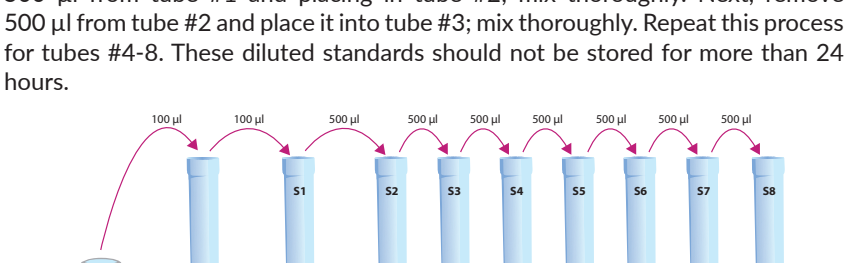


Figure 4. Preparation of the testosterone standards

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 ELISA Buffer (1X).

OR

500 dtn Testosterone AChE Tracer (480-well kit; Item No. 482700): Reconstitute with 30 ml ELISA Buffer (1X).

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). *NOTE: Do not store tracer with dye.*

Testosterone ELISA Antiserum

Reconstitute the Testosterone ELISA Antiserum as follows:

100 dtn Testosterone ELISA Antiserum (96-well kit; Item No. 482702): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn Testosterone ELISA Antiserum (480-well kit; Item No. 482702): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted Testosterone ELISA 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). *NOTE: Do not store antiserum with dye.*

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), and three 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 23, for more details). We suggest you record the contents of each well on the template sheet provided (see page 34).

	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 5. 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. **ELISA Buffer**

   Add 100 µl ELISA Buffer (1X) to NSB wells. Add 50 µl ELISA Buffer (1X) to B_0 wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for ELISA Buffer (1X) in the NSB and B_0 wells (*i.e.*, add 50 µl culture medium to NSB and B_0 wells and 50 µl ELISA Buffer (1X) to NSB wells).

2. **Testosterone ELISA 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 TA and the Blk wells.

5. **Testosterone ELISA Antiserum**

   Add 50 µl to each well *except* the TA, the NSB, and the Blk wells.

Well	ELISA Buffer	Standard/ Sample	Tracer	Antiserum
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 2. Pipetting summary

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (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 TA 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.*
3. Read the plate at a wavelength between 405 and 420 nm.

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 analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) 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 TA values are not used in the standard curve calculations. Rather they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme/substrate system.

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 four-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.

Performance Characteristics

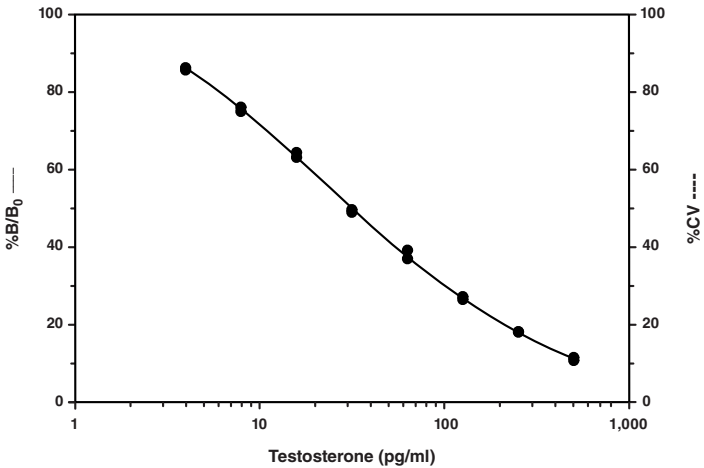
Sample Data

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)	Blk-Subtracted		NSB-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 3. Typical results



Assay Range = 3.9-500 pg/ml
Sensitivity (defined as 80% B/B_0) = 6 pg/ml
Mid-point (defined as 50% B/B_0) = 35 pg/ml
Lower Limit of Detection (LLOD) = 3 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer (1X).

Figure 6. 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 29 and in the table below.

Dose (pg/ml)	%CV* Intra-assay precision	%CV* Inter-assay precision
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 4. Intra- and inter-assay precision
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

Cross Reactivity:

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 5. Cross reactivity of the Testosterone ELISA

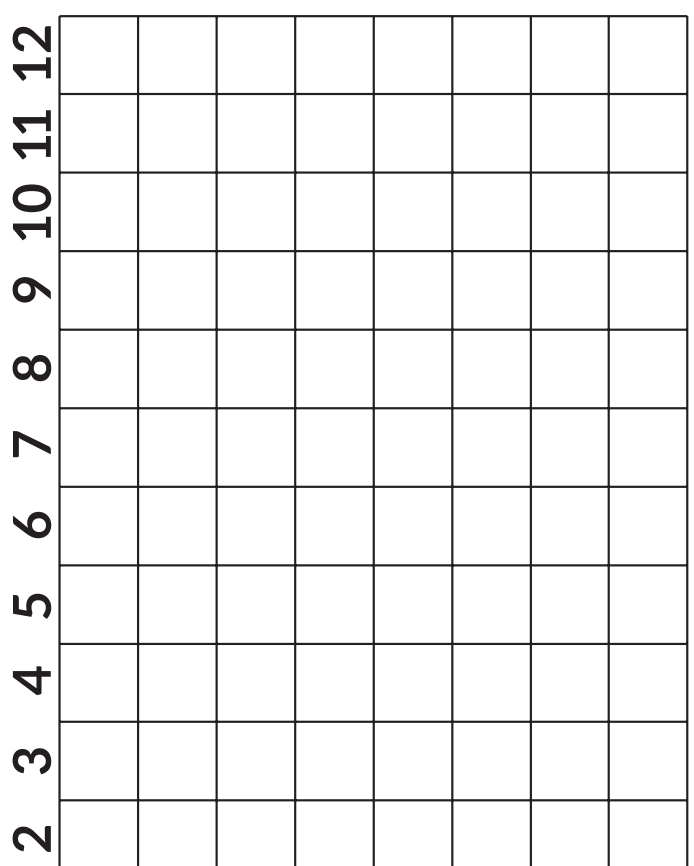
RESOURCES

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 (>10% of B_0)	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 ELISA[3]
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. 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).
3. Maxey, K.M., Maddipati, K.R., and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**, 116-120 (1992).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©03/27/2026, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.