Testosterone high sensitivity ELISA kit

Catalog # ADI-901-176
5 x 96 Well Enzyme-Linked Immunosorbent Assay Kit
For use with serum, plasma, and urine

Store at 4°C.

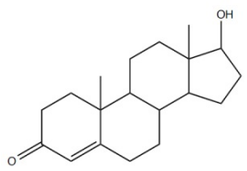
Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

Table of Contents

Introduction

Testosterone is one of the most important androgens secreted into the bloodstream and is synthesized from pregnenolone which is itself formed from cholesterol[1-4]. In adult humans approximately 5 mg of testosterone are synthesized per day and circulate in plasma predominately bound to proteins, including specific sex hormone binding globulin (SHBG) and nonspecific proteins such as albumin. It is believed that the bioavailable testosterone includes the free steroid and the albumin bound steroid
and these equal about 35% of the total testosterone5. Both testosterone and SHBG exhibit rhythmic variations. In serum, testosterone concentration peaks between 4am and 8am, while SHBG concentration is effected by such factors as posture[5,6]. Testosterone is the main androgen secreted by the Leydig cells of the testes and effects both primary and secondary sexual development such as muscle mass and sex drive[7,8].



Principle

1. Standards and samples are added to wells coated with a GxM IgG antibody. A blue solution of Testosterone conjugated to alkaline phosphatase is then added, followed by a yellow solution of mouse monoclonal antibody to testosterone.
2. During a simultaneous incubation at room temperature the antibody binds in a competitive manner, the testosterone in the sample or conjugate. The plate is washed, leaving only bound testosterone.
3. pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the testosterone conjugate.
4. Stop solution is added. The yellow color is read at 405 nm. The amount of signal is indirectly proportional to the amount of testosterone in the sample.

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.

Activity of conjugate is affected by concentrations of chelators > 10mM (such as EDTA and EGTA).

Stop solution is caustic. Keep tightly capped.

Materials Supplied

1. **Assay Buffer Concentrate Low BSA**
   27 mL, Product No. 80-2088
   Tris buffer containing proteins and sodium azide
2. **Testosterone Standard**
   3 x 0.5 mL, Product No. 80-0430
   A solution of 50,000 pg/mL Testosterone
3. **Goat anti-Mouse IgG Microtiter Plate**
   Five plates of 96 wells, Product No. 80-0050
   A clear plate of break-apart strips coated with a goat anti-mouse polyclonal antibody
4. **Testosterone Antibody**
   27 mL, Product No. 80-2299
   A yellow solution of a monoclonal antibody to Testosterone
5. **Testosterone Conjugate**
   27 mL, Product No. 80-2300
   A blue solution of Testosterone conjugated to alkaline phosphatase
6. **Wash Buffer Concentrate**
   100 mL, Product No. 80-1287
   Tris buffered saline containing detergents
7. **pNpp Substrate**
   100 mL, Product No. 80-0076
   A solution of p-nitrophenyl phosphate
8. **Stop Solution**
   27 mL, Product No. 80-0248
   A solution of trisodium phosphate in water
9. **Testosterone Complete Assay Layout Sheet**
   1 each, Product No. 30-0288
10. **Plate Sealer**
    5 each, Product No. 30-0012

Storage

All components of this kit are stable at 4°C until the kit's expiration date.

Materials Needed but Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 50 µL and 200 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Microplate shaker
7. Lint-free paper toweling for blotting
8. Microplate reader capable of reading at 405 nm
9. 200 mg C_{18} solid phase system columns (Burdick & Jackson recommended)
10. Vacuum manifold
11. Speedvac
12. 100% Methanol
13. Diethyl Ether
14. Data reduction software capable of analyzing data, preferably with a 4 parameter logistic curve fit.

Sample Handling

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Samples must be stored at or below -20˚C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

For an accurate determination of testosterone concentration in serum plasma and urine samples, extract samples prior to assaying. A minimum 1:4 dilution is required for all samples after extraction. These are the minimum dilutions required to remove matrix interference in the assay.

Samples with low levels of testosterone may be concentrated during the extraction procedure. For example, extracting 1 mL of sample and reconstituting with 0.25 mL of the assay buffer would concentrate the sample 4 times.

Either liquid-liquid extraction or solid phase extraction methods may be used. Solid phase extraction allows for higher throughput sample processing with the use of a vacuum manifold and speedvac.

Solid Phase Extraction

Materials Needed:

1. Testosterone standard to allow extraction efficiency to be accurately determined. An extraction efficiency protocol is available on our website.
2. 200 mg C_{18} solid phase system columns (Burdick & Jackson recommended)
3. Vacuum manifold
4. Speedvac
5. 100% Methanol
6. Diethyl Ether
7. dH_20

Procedure:

1. Condition 200 mg C_{18} solid phase system columns on a vacuum manifold by passing 5-10 mL of 100% methanol through the columns, followed by 5-10 mL of dH_2O.
2. Apply serum and plasma samples.
3. Wash columns with 5-10 mL dH_2O. Allow water to drain completely from columns until dry.
4. Elute samples with 2 mL of diethyl ether.
5. Dry samples down in a speedvac for 2-3 hrs.
6. Rehydrate samples at room temperature in the assay buffer. A minimum of 250 µL of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement.

Liquid-Liquid Extraction

Materials Needed:

1. Testosterone standard to allow extraction efficiency to be accurately determined. An extraction efficiency protocol is available on our website.
2. Diethyl Ether
3. Speedvac
4. dH_20

Procedure:

1. Add diethyl ether to serum or plasma samples at a 5:1 (v/v) ether:sample ratio.
2. Mix solutions by vortexing for 2 minutes.
3. Allow phases to separate for 2 minutes.
4. Transfer organic phase to a glass test tube containing 1 mL dH_2O.
5. Vortex the mixture for 2 minutes, and allow phases to separate for 2 minutes.
6. Transfer organic phase to a clean glass test tube, and dry samples down using a speed vac for 2-3 hours.
7. Rehydrate samples at room temperature in the assay buffer. A minimum of 250 µL of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement

Tip: Place samples in the freezer for a few minutes to help the layers separate.

Sample Recoveries

Testosterone was spiked into samples at high, medium and low concentrations, prior to liquid-liquid extractions. Endogenous testosterone was subtracted from the spiked values and the average recovery in each of the spiked matrices was compared to the recovery of identical spikes in extracted assay buffer. The mean and the range percent recovery at the three concentrations are indicated below for each matrix.

Sample Matrix	Dilution	Spike Concentration	Recovery of Spike (Range)
Human Plasma (n=5)	1:16	1000 pg/mL 200 pg/mL 50 pg/mL	84% (78-96%) 86% (55-117%) 118% (62-141%)
Human Serum (n=5)	1:16	1000 pg/mL 200 pg/mL 50 pg/mL	84% (70-93%) 97% (66-145%) 111% (63-115%)
Human Urine (n=1)	1:16	1000 pg/mL 200 pg/mL 50 pg/mL	111% 82% 117%

Reagent Preparation

Bring all reagents to room temperature for at least 30 minutes prior to opening.

1. **Assay Buffer**

   Prepare the Assay Buffer by diluting 10mL of the supplied Assay Buffer Concentrate with 90mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

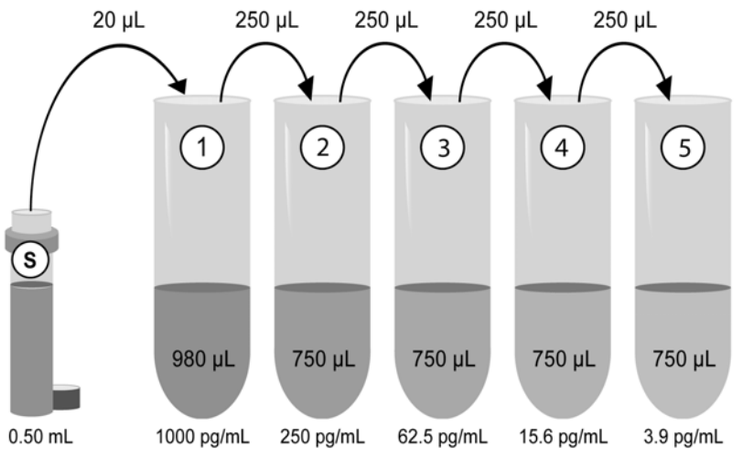
2. **Wash Buffer**

   Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer Concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

3. **Testosterone Standard**

   Allow the 50,000 pg/mL Testosterone standard to come to room temperature and vortex prior to use. Label five 12 x 75mm tubes #1 through #5. Pipet 1mL assay buffer into tube #1. Pipet 750 µL assay buffer into tubes #2 through #5. Remove 20 µL of assay buffer from tube #1.



of the 50,000 pg/mL standard stock to tube #1 and vortex thoroughly. Add 250 µL from tube #1 and add to tube #2. Vortex thoroughly. Continue this for tubes #3 through #5.

Diluted standards should be used within 60 minutes of preparation. The concentrations of Testosterone in the tubes are labeled above.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

All standards and samples should be run in duplicate.

Pipet the reagents to the sides of the wells to avoid possible contamination.

1. Pipet 150 µL of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100 µL of the assay buffer into the Bo (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #5 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.
5. Pipet 50 µL of the blue conjugate into each well except the TA and Blank wells.
6. Pipet 50 µL of the yellow antibody into each well except the Blank, TA, and NSB wells.

   Note: Every well used should be green in color except the NSB wells which should be blue. The Blank and TA wells are empty at this point and have no color.
7. Seal the plate. Incubate at room temperature with shaking (~500 rpm*) for two hours.
8. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

9. Pipet 5 µL of the blue conjugate to the TA wells.
10. Add 200 µL of the substrate solution into each well.
11. Incubate for 1 hour at room temperature with shaking.
12. Pipet 50 µL stop solution into each well.
13. After blanking the plate reader against the substrate blank, read optical density at 405 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

* The optimal speed for each shaker will vary and may range from 120-700 rpm. The speed must be set to ensure adequate mixing of the wells, but not so vigorously that the contents of the wells splash out and contaminate other wells.

Calculation of Results

Several options are available for the calculation of the concentration of Testosterone in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of Testosterone can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   $$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot the Percent Bound (B/Bo) versus concentration of Testosterone for the standards. Approximate a straight line through the points. The concentration of Testosterone of the unknowns can be determined by interpolation.

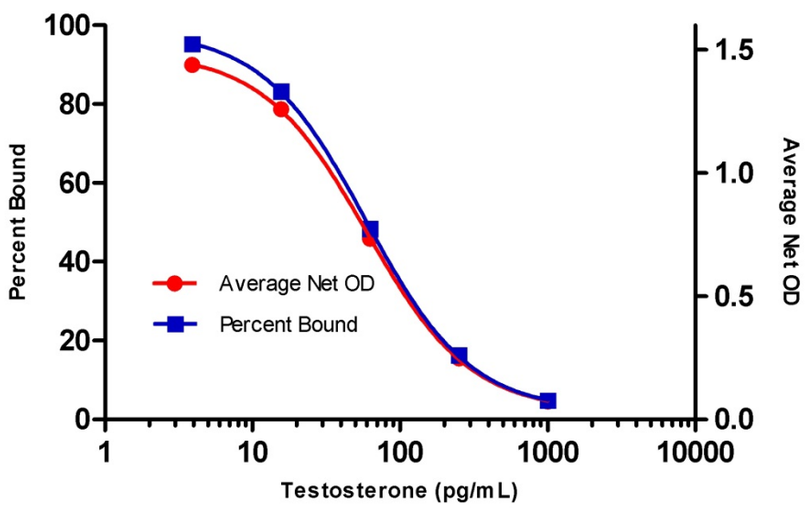
Make sure to adjust sample concentrations by the dilution or concentration factor used during sample preparation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Average Net OD	Percent Bound	Testosterone (pg/mL)
TA	0.436	---	---
NSB	0.095	0%	---
Bo	1.511	100%	0
S1	0.074	4.8%	1000
S2	0.249	16.3%	250
S3	0.734	48.4%	62.5
S4	1.259	83.2%	15.6
S5	1.439	95.2%	3.9



Performance Characteristics

Specificity

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of 100, 10, 1, and 0.1 times the high standard. These samples were then measured in the assay.

Compound	Cross Reactivity
Androstenedione	16.4%
19-Hydroxytestosterone	7.6%
Dihydrotestosterone	2.7%
Progesterone	0.3%
Androsterone	0.2%
DHEA	0.22%
Estrone	0.09%
Estradiol	<0.004%
Estriol	<0.004%
Epitestosterone	<0.004%
Hydrocortisone-21 hemi-succinate	<0.004%

Sensitivity

The sensitivity or limit of detection of the assay is 2.6 pg/mL. The sensitivity was determined by interpolation at 2 standard deviations below the mean signal at maximal binding (0 pg/mL) using data from 7 standard curves.

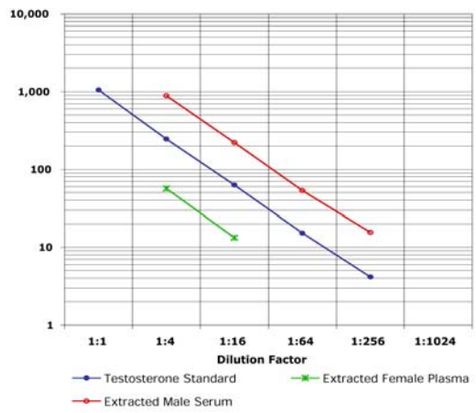
Linearity

Human serum , plasma and urine samples were serially diluted 1:4 in the kit assay buffer and measured in the assay. The results are shown in the table below.

	Average % of Expected		
Dilution	Serum	Plasma	Urine
Neat	---	---	---
1:4	104	103	108
1:16	104	94	100
1:64	100	106	99
1:256	116	100	87

Parallelism

Dose-response curves from extracted male serum and extracted female plasma diluted into assay buffer were compared to the Testosterone standard curve. The parallel response indicates the standard effectively mimics the native protein.



Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing Estradiol-17β in a single assay.

pg/mL	%CV
374.9	4.1
79.70	2.3
43.05	3.6

Inter-assay precision was determined by measuring buffer controls (n=13) of varying Estradiol-17β concentrations in multiple assays over several days.

pg/mL	%CV
388.8	9.9
86.8	10.2
43.0	7.4

References

1. M.L. Dufua, et al., J. Steriod Biochem., (1984) 20: 161-173.
2. P.F. Hall, "The Physiology of Reproduction", (1988) Raven Press. 975-998.
3. J.E. Griffen & J.D. Wilson, "Williams Textbook of Endocrinology 8th Ed.", (1992) W. B. Saunders. 799-852.
4. F.Z. Stanczyk, "Infertility, Contraception & Reproductive Endocrinology", (1991) Blackwell. 53-76.
5. R.J. Santen, "Reproductive Endocrinology", (1992) W. B. Saunders. 739-794.
6. J. Valero-Polito & X. Fuentes-Arderiu, Eur. J. Clin. Biochem., (1996) 34: 455-462.
7. J. Imperato-McGinley, Science, (1974) 186: 1213-1215.
8. J.D. Wilson, Science, (1981) 211: 1278-1284.

Notes

Notes

MSDS (Material Safety Data Sheet) available online

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

www.enzolifesciences.com

Enabling Discovery in Life Science®

Global Headquarters
Enzo Life Sciences Inc.
10 Executive Blvd
Farmingdale, NY 11735
(p) 1-800-942-0430
(f) 1-631-694-7501
(e) info-usa@enzolifesciences.com

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lause / Switzerland
(p) +41/0 61 926 89 89
(f) +41/0 61 926 89 79
(e) info-ch@enzolifesciences.com

Please visit our website at www.enzolifesciences.com for additional contact information.

Catalog Number: 25-0725