

ARBOR ASSAYS®
Interactive Assay Solutions™



DetectX®

Testosterone Enzyme Immunoassay Kit

1 or 5 Strip Well Plates Catalog Number K080-H1/H5

Species Independent

Sample Types Validated:

Serum, Plasma, Urine, and Dried Fecal Extracts

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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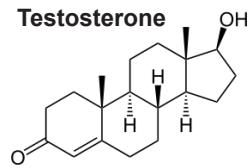
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BACKGROUND

Testosterone, C₁₉H₂₈O₂, (4-Androsten-17 β -ol-3-one) is a steroid hormone from the androgen group and is found in mammals, reptiles, birds, and other vertebrates^{1,2}. In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid.

In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair³. In the absence of testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage. In addition, testosterone is essential for health and well-being⁴ as well as the prevention of osteoporosis⁵. On average, an adult human male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone⁶. Testosterone plays a significant role in glucose homeostasis and lipid metabolism. Low testosterone levels in males can be associated with the metabolic syndrome, Hypogonadism, a clustering of risk factors predisposing to type 2 diabetes mellitus, atherosclerosis and cardiovascular morbidity and mortality⁷. The main components of the syndrome are visceral obesity, insulin resistance, glucose intolerance, raised blood pressure and dyslipidemia (elevated triglycerides, low levels of high-density lipoprotein cholesterol), and a pro-inflammatory and thrombogenic state. Cross-sectional epidemiological studies have reported a direct correlation between plasma testosterone and insulin sensitivity, and low testosterone levels are associated with an increased risk of type 2 diabetes, dramatically illustrated by androgen deprivation in men with prostate carcinoma. While age-related decline of testosterone is the primary associated factor for lower levels of free testosterone the sexual hormone-binding globulin (SHBG) increases later in life which also contribute to lower levels⁷.

Testosterone is observed in most vertebrates. Fish make a slightly different form called 11-ketotestosterone⁸. Its counterpart in insects is ecdysone⁹. These ubiquitous steroids suggest that sex hormones have an ancient evolutionary history¹⁰.



1. Cox, R. M., & John-Adler, H. B. (2005). Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. *Journal of Experimental Biology*, 208(24), 4679–4687.
2. Reed, W. L., et al. (2006). Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness. *The American Naturalist*, 167(5), 667–683.
3. Mooradian, A. D., et al. (1987). Biological actions of androgens. *Endocrine reviews*, 8(1), 1–28.
4. Bassil, N., et al. (2009). The benefits and risks of testosterone replacement therapy: a review. *Therapeutics and clinical risk management*, 5, 427–448.
5. Tuck, S. P., & Francis, R. M. (2009). Testosterone, bone and osteoporosis. In T. H. Jones (Ed.). *Advances in the management of testosterone deficiency* (vol. 37, 123–132). Sheffield, GB: Karger Publishers.
6. Dabbs, M., & Dabbs, J. M. (2000). *Heroes, rogues, and lovers: Testosterone and behavior*. New York, NY: McGraw-Hill.
7. Nelson, R. J. (2005). *An introduction to behavioral endocrinology* (3rd ed.). Sunderland, MA: Sinauer Associates.
8. Traish AM, Miner MM, Morgentaler A, et al. Testosterone deficiency. Am J Med. 2011;124(7):578–587.
9. De Loof, A. (2006) Ecdysteroids: The overlooked sex steroids of insects? Males: The black box. *Insect Science*, 13(5), 325–338.
10. Mechoulam, R., et al. (1984). Estrogens in insects. *Experientia*, 40, 942–944.

ASSAY PRINCIPLE

The DetectX® Testosterone Immunoassay Kit uses a specifically generated antibody to quantitatively measure testosterone and its metabolites in serum, plasma, urine and dried fecal extracts. Please read the complete kit insert before performing this assay. A testosterone standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture sheep antibodies. A testosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to testosterone to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound testosterone-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the testosterone in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
11-Ketotestosterone ELISA Kits	K079-H1/H5
Androstenedione ELISA Kits	K070-H1/H5
Corticosterone ELISA Kits	K014-H1/H5
Cortisol Enzyme Immunoassay Kits	K003-H1/H5
Cortisone ELISA and Chemiluminescent ELISA Kits	K017-H1/H5, K017-C1/C5
Dehydro-epiandrosterone sulfate (DHEA-S) ELISA Kits	K054-H1/H5
Epiandrosterone ELISA Kits	K063-H1/H5
Estradiol Non-Invasive & Serum ELISA Kits	K030-H1/H5, KB30-H1/H5
Estrone ELISA Kits	K031-H1/H5
PGFM (13,14-dihydro-15-keto-Prostaglandin F2alpha) ELISA Kits	K022-H1/H5
Pregnanediol 3-Glucuronide (PDG) ELISA Kits	K037-H1/H5
Progesterone Metabolites ELISA Kits	K068-H1/H5
Testosterone ELISA Kits	K032-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.

Kit K080-H1 or -H5 1 or 5 Each Catalog Number X061-1EA, 1 x 8 Strip Well

Testosterone Standard

Testosterone at 200,000 pg/mL in a special stabilizing solution.

Kit K080-H1 or -H5 70 µL or 350 µL Catalog Number C113-70UL or -350UL

DetectX® Testosterone Antibody

A sheep monoclonal antibody specific for testosterone

Kit K080-H1 or -H5 3 mL or 13 mL Catalog Number C293-3ML or -13ML

DetectX® Testosterone Conjugate **Must be stored at $\leq -20^{\circ}\text{C}$.**

A testosterone-peroxidase conjugate in a special stabilizing solution.

Kit K080-H1 or -H5 3 mL or 13 mL Catalog Number C112-3ML or -13ML

Assay Buffer Concentrate

A 5X concentrate that should be diluted with deionized or distilled water.

Kit K080-H1 or -H5 28 mL or 55 mL Catalog Number X065-28ML or -55ML

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.

Kit K080-H1 or -H5 30 mL or 125 mL Catalog Number X007-30ML or -125ML

TMB Substrate

Kit K080-H1 or -H5 11 mL or 55 mL Catalog Number X019-11ML or -55ML

Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**

Kit K080-H1 or -H5 5 mL or 25 mL Catalog Number X020-5ML or -25ML

Plate Sealer

Kit K080-H1 or -H5 1 or 5 Each Catalog Number X002-1EA

Dissociation Reagent

Dissociation Reagent is to be used only with Serum and Plasma samples.

Kit K080-H1 or -H5 1 or 5 Each Catalog Number X154-1ML or -5ML

STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C .

Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the Testosterone Conjugate. This must be stored at $\leq -20^{\circ}\text{C}$.**

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes.

Repeater pipet with disposable tips capable of dispensing 25, 50, and 100 μ L.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The testosterone standard used for this kit is an anabolic steroid and may have a number of known and unknown biological actions. Care should be taken in handling this material.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers' Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for serum, plasma, urine, and dried fecal samples. Samples containing visible particulate should be centrifuged prior to using. Testosterone is identical across all species and we expect this kit to measure testosterone from all sources. The end user should evaluate recoveries of testosterone in other sample matrices being tested.

SAMPLE PREPARATION

Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total testosterone concentration in serum or plasma. **Dissociation Reagent is to be used only with Serum and Plasma samples.**

Serum and Plasma Samples

Allow the Dissociation Reagent to warm completely to Room Temperature before use. We suggest pipetting 10 μ L of Dissociation Reagent into 1 mL Eppendorf tube. Add 10 μ L of serum or plasma to the Dissociation Reagent in the tube, vortex periodically while incubating at room temperature for 15 minutes or longer.

Dilute this 1:2 mixture of serum or plasma and dissociation reagent at least 1:18 with 1X Assay Buffer before running in the assay. The final serum and plasma dilutions should be \geq 1:36, as this is the minimum dilution to remove matrix effects of the dissociation reagent in the assay.

NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

Saliva Samples

Saliva samples should be diluted \geq 1:4 with 1X Assay Buffer prior to running in the assay. See our Saliva Sample Handling instructions at: www.ArborAssays.com/assets/saliva-sample-protocol.pdf.

Dried Fecal Samples

We have a detailed Extraction Protocol available on our website at: www.ArborAssays.com/resources/#protocols. The ethanol concentration in the final Assay Buffer dilution added to the well should be < 5%.

Urine Samples

Urine samples should be diluted at \geq 1:8 with 1X Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection Kits, K002-H1 and K002-H5. Use all samples within 2 hours of preparation.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

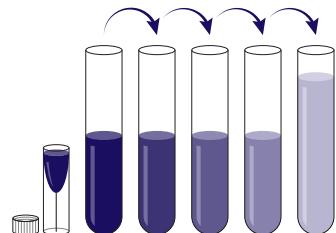
Dilute Assay Buffer Concentrate 5-fold by adding one part of the concentrate to four parts of deionized water. The 1X Assay Buffer is stable at 4°C for 3 months.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. The 1X Wash Buffer is stable at room temperature for 3 months.

Standard Preparation

Label test tubes as Inter #1 and Stds #1 through #8. Pipet 475 µL of 1X Assay Buffer into tube Inter #1, 450 µL into Std tube #1, and 250 µL into tube #2 through #8. **The testosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 25 µL of the testosterone stock solution to Inter #1 and vortex completely. Then take 250 µL of the testosterone solution in Std #1 and add it to Std tube #2 and vortex completely. Continue to serially dilute for tubes #3 through #8. The concentration of testosterone in tubes #1 through #8 will be 1,000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 pg/mL.



Use all Standards within 2 hours of preparation.

	Inter 1	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
1X Assay Buffer (µL)	475	450	250	250	250	250	250	250	250
Addition	Stock	Inter 1	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	25	50	250	250	250	250	250	250	250
Final Conc (pg/mL)	10,000	1,000	500	250	125	62.5	31.25	15.63	7.81

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine testosterone concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4 °C.
2. Pipet 50 µL of samples or standards into wells in the plate.
3. Pipet 75 µL of 1X Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of 1X Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® Testosterone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Testosterone Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 20% lower.
8. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate testosterone concentration for each sample.

NOTE: *If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.*

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

www.myassays.com/arbor-assays-detectx-testosterone-assay

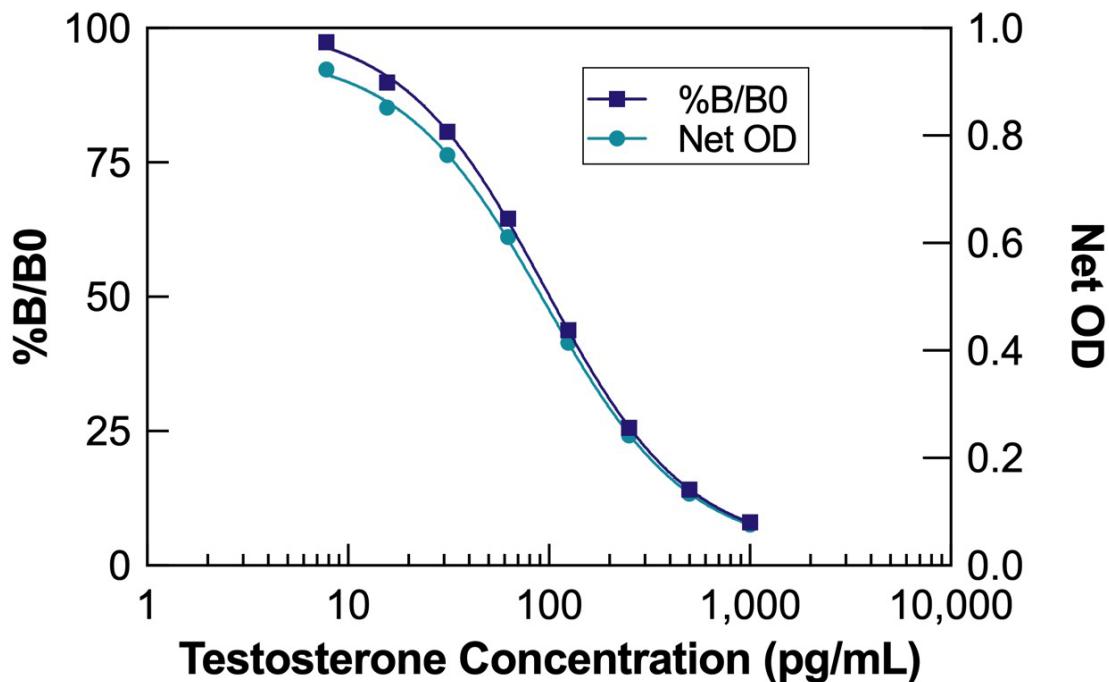
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Testosterone Conc. (pg/mL)
NSB	0.081	0.00	-	-
Standard 1	0.156	0.076	8.0	1,000.0
Standard 2	0.215	0.134	14.1	500.0
Standard 3	0.323	0.242	25.6	250.0
Standard 4	0.495	0.415	43.8	125.0
Standard 5	0.692	0.611	64.5	62.5
Standard 6	0.845	0.764	80.7	31.25
Standard 7	0.932	0.852	89.9	15.630
Standard 8	1.003	0.923	97.4	7.810
B0	1.028	0.947	100.0	0
Sample 1	0.430	0.350	36.9	159.6
Sample 2	0.619	0.539	56.9	80.1

Always run your own standard curve for calculation of results. Do not use this data.

Conversion Factor: 100 pg/mL of testosterone is equivalent to 346.7 pM.

Typical Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

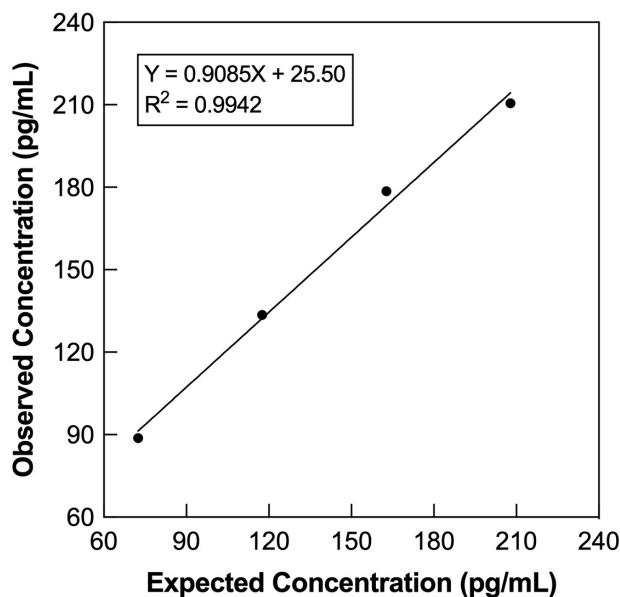
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two standard deviations from the B0 along the standard curve. **Sensitivity was determined as 6.53 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration sample. **Limit of Detection was determined as 9.48 pg/mL.**

Linearity

Linearity was determined by taking two urine samples diluted with 1X Assay Buffer, one with a low diluted testosterone level of 27.3 pg/mL and one with a higher diluted level of 253.0 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	207.86	210.5	101.3
60%	40%	162.72	178.5	109.7
40%	60%	117.58	133.5	113.5
20%	80%	72.44	88.8	122.6
Mean Recovery				111.8%



Intra Assay Precision

Three spiked samples were diluted with 1X Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Testosterone concentrations were:

Sample	Testosterone Conc. (pg/mL)	%CV
1	151.5	4.1
2	74.8	4.9
3	36.9	7.0

Inter Assay Precision

Three spiked samples were diluted with 1X Assay Buffer and run in duplicates in eleven assays run over multiple days by seven operators. The mean and precision of the calculated Testosterone concentrations were:

Sample	Testosterone Conc. (pg/mL)	%CV
C1	226.4	9.9
C2	155.8	10.5
C3	80.4	10.3

SAMPLE VALUES

Sixteen serum and four plasma samples from humans were tested in the assay at various dilutions. Adjusted neat concentrations of Testosterone ranges from 317.0 to 8440.5 pg/mL. Seven urine samples from various species were tested in the assay. Adjusted neat concentrations of Testosterone ranged from 51.2 to 6157.6 pg/mL. Seven saliva samples from humans were tested in the assay. Adjusted neat concentrations of Testosterone ranged from 6.8 to 328.0 pg/mL. Fecal samples from four different species were extracted and tested in the assay. Adjusted neat concentrations of Testosterone ranged from 22.24 to 149.92 pg/mg dried feces.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)	Steroid	Cross Reactivity (%)
Testosterone	-	Aldosterone	< 0.01%
11-Ketotestosterone	10.53%	Pregnenolone	< 0.01%
Dihydrotestosterone	3.24%	Progesterone	< 0.01%
Estradiol	0.430%	Cortisol	< 0.01%
DHEA	0.040%	Corticosterone	< 0.01%
Cholesterol	< 0.01%	Cortisone	< 0.01%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with ELISA kits for wildlife conservation research.

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