ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

11-Ketotestosterone
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K079-H1
5 Plate Kit Catalog Number K079-H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts, Urine, and Extracted Serum/Plasma

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

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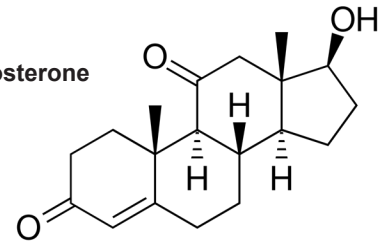
BACKGROUND

Androgenic hormones, such as testosterone, affect the growth, size, and reproduction of many male organisms. In telost fish, along with testosterone, 11-ketotestosterone plays a significant role by inducing reproductive characteristics in both males and females.[1] In certain male fish, 11-ketotestosterone levels increase during spermatogenesis in spawning season,[2] while in some female fish, 11-ketotestosterone increases prior to yolk deposition to regulate ovarian development.[3]

11-Ketotestosterone



The presence and involvement of 11-ketotestosterone in other species such as humans have only recently been established. In contrast to fish, primate serum 11-ketotestosterone concentrations were not significantly different in male and female, despite males having significantly higher circulating testosterone.[4] This suggests that 11-ketotestosterone production in these species may not be testis-dependent and primarily originates from adrenal-derived 11-oxyandrogen precursors.[4] Recent studies have discovered there is more 11-ketotestosterone than its precursors, androstenedione and testosterone, in prepubertal children and postmenopausal women compared to men.[5] This highlights the potential use of 11-ketotestosterone as a clinical biomarker to screen adrenal androgen excess in disease conditions such as polycystic ovary syndrome (PCOS) and hirsutism in women.[6]

1. Prat, F., *et al.* (1990). Seasonal changes in plasma levels of gonadal steroids of sea bass, *Dicentrarchus labrax L. General and Comparative, Endocrinology, 78*(3), 361–373.
2. Nagahama, Y., *et al.* (1994). The onset of spermatogenesis in fish. In *Germline Dev. (Series: Ciba Foundation Symposia)*. (Vol. 182, 255–270.)
3. Wang, W., *et al.* (2020). Effects of 11-ketotestosterone on development of the previtellogenic ovary in the sterlet, Acipenser ruthenus. *Frontiers in Endocrinology, 11*, 15.
4. Rege, J., *et al.* (2019). Circulating 11-oxygenated androgens across species. *The Journal of Steroid Biochemistry and Molecular Biology, 190*, 242–249.
5. Turcu, A. F., *et al.* (2020). 11-oxygenated androgens in health and disease. *Nature Reviews Endocrinology, 16*(5), 284–296.
6. O'Reilly, M. W., *et al.* (2017). Oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. *The Journal of Clinical Endocrinology & Metabolism, 102*(3), 840–848.

ASSAY PRINCIPLE

The DetectX® 11-Ketotestosterone Immunoassay Kit uses a specifically generated antibody to measure 11-ketotestosterone in urine or in extracted fecal, serum, and plasma samples. This kit is not recommended for serum or plasma samples without extraction. Please read the complete kit insert before performing this assay. An 11-ketotestosterone 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 rabbit antibodies. An 11-ketotestosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal 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 11-ketotestosterone-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 11-ketotestosterone 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.
Androstenedione ELISA Kits	K070-H1/H5
Corticosterone ELISA Kits	K014-H1/H5
Cortisol Enzyme Immunoassay Kits (Strip Wells and Whole Plate)	K003-H1/H5/H1W/H5W
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 goat anti-rabbit IgG.

Kit K079-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

11-Ketotestosterone Standard
11-Ketotestosterone at 100,000 pg/mL in a special stabilizing solution.

Kit K079-H1 **or** -H5	70 µL **or** 350 µL	Catalog Number C292-70UL **or** -350UL

DetectX® 11-Ketotestosterone Antibody
A rabbit polyclonal antibody specific for 11-ketotestosterone

Kit K079-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C290-3ML **or** -13ML

DetectX® 11-Ketotestosterone Conjugate
11-Ketotestosterone-peroxidase conjugate in a special stabilizing solution.

Kit K079-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C291-3ML **or** -13ML

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

Kit K079-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 K079-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K079-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K079-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K079-H1 **or** -H5	1 **or** 5 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes.

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

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

Diethyl ether or ethyl acetate for extraction of serum and plasma.

Ethanol for extraction of fecal material.

A Speedvac/centrifugal concentrator or N_2 gas and gas manifold for evaporation.

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 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 extracted dried fecal, urine, and extracted serum and plasma samples. Samples containing visible particulate should be centrifuged prior to using. 11-Ketotestosterone can be assayed in solid sample types or in serum and plasma samples by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols

11-Ketotestosterone is identical across all species and we expect this kit to measure 11-ketotestosterone from all sources. The end user should evaluate recoveries of 11-ketotestosterone in other sample matrices being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

We have 3 detailed Extraction Protocols available on our website at: www.ArborAssays.com/resources/#protocols as a PDF file entitled "Steroid Serum/Plasma Extraction Protocol". We would recommend the following protocol for serum and plasma.

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. Allow ether layer to separate for 5 minutes.
3. Freeze samples in a dry ice/ethanol bath and pipet off the ether solution from the top ofthe sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions.
4. Dry pooled ether samples down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20°C.
5. Redissolve samples at room temperature in the Assay Buffer. A minimum of 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

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 least 1:8 with the provided 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

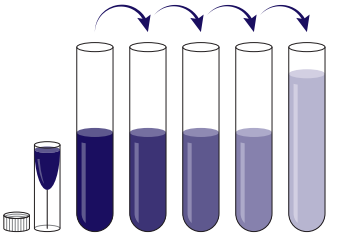
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer

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

Standard Preparation

Label test tubes as #1 through #7. Pipet 490 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #7. **The 11-ketotestosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 µL of the stock solution to tube #1 and vortex completely. Take 200 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of 11-ketotestosterone in tubes 1 through 7 will be 2,000, 800, 320, 128, 51.2, 20.5 and 8.19 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	**490**	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**10**	200	200	200	200	200	200
Final Conc (pg/mL)	2,000	800	320	128	51.2	20.5	8.19

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine 11-ketotestosterone 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 Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® 11-Ketotestosterone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® 11-Ketotestosterone 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 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: https://myassays.com/arbor-assays-detectx-11-ketotestosterone-enzyme-immunoassay-kit.assay

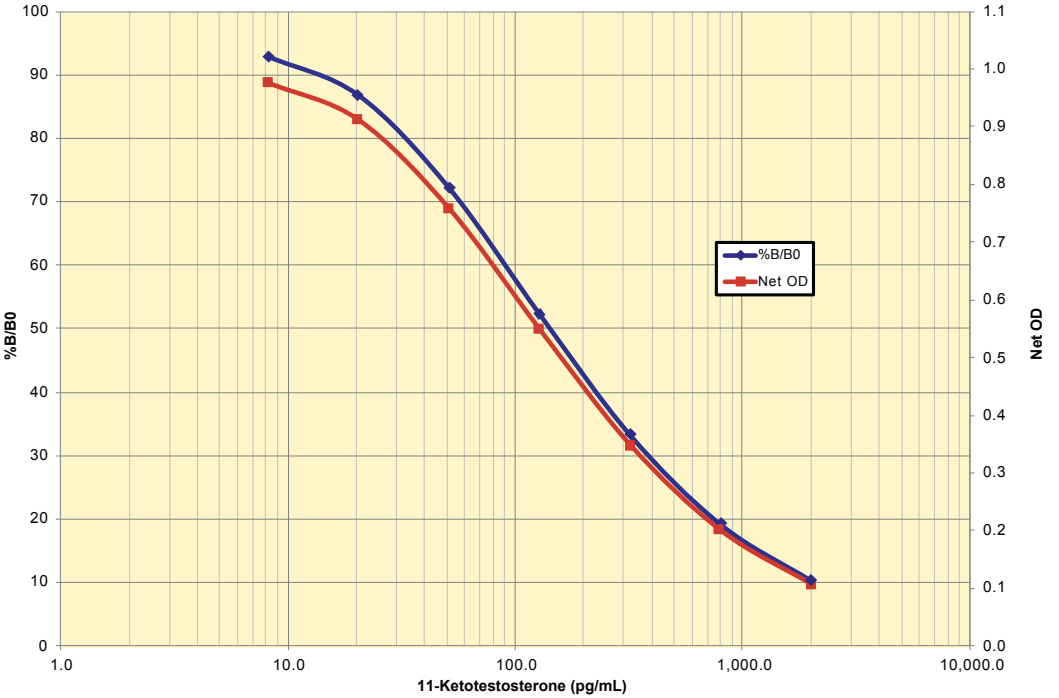
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	11-Ketotestosterone Conc. (pg/mL)
NSB	0.092	0	-	-
Standard 1	0.200	0.108	10.3	2,000.0
Standard 2	0.293	0.201	19.1	800.0
Standard 3	0.441	0.349	33.2	320.0
Standard 4	0.641	0.549	52.3	128.0
Standard 5	0.850	0.758	72.2	51.2
Standard 6	1.004	0.912	86.9	20.5
Standard 7	1.068	0.976	93.0	8.19
B0	1.142	1.050	100	0
Sample 1	0.453	0.361	34.4	298.4
Sample 2	0.707	0.615	58.6	99.3

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

Conversion Factor: 100 pg/mL of 11-ketotestosterone is equivalent to 330.7 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

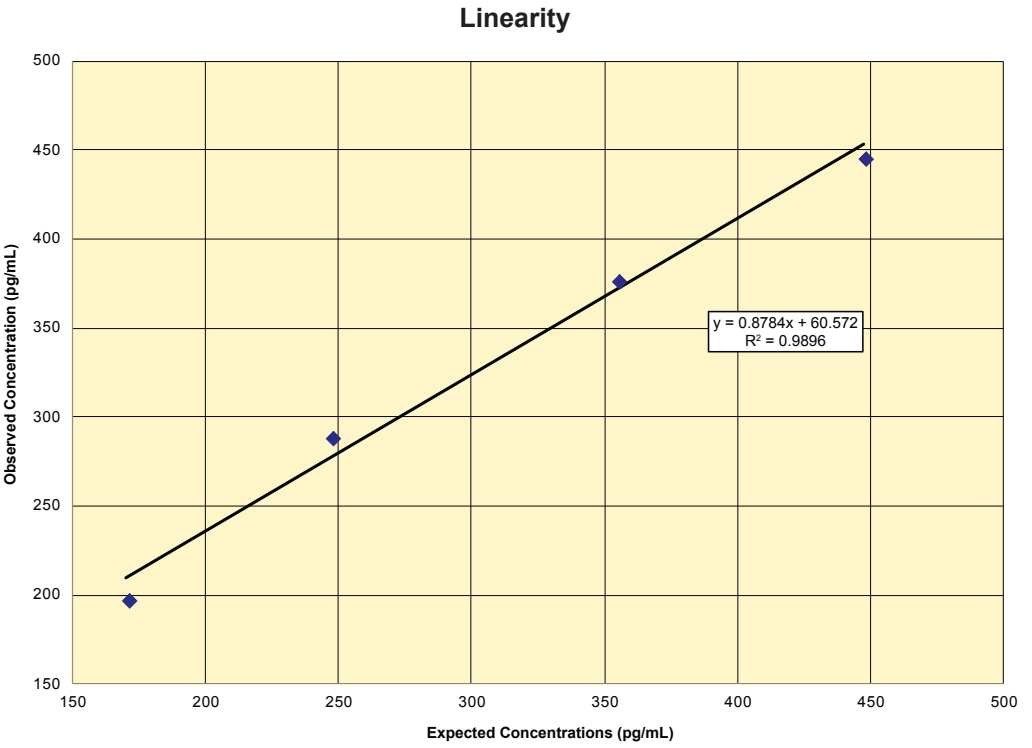
Sensitivity was calculated by comparing the OD's for 20 wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 1.85 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for 20 runs for each of the zero standard and a low concentration human urine sample. **Limit of Detection was determined as 15.9 pg/mL.**

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted 11-ketotestosterone level of 77.9 pg/mL and one with a higher diluted level of 539.6 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%	447.3	446.3	99.8
60%	40%	354.9	377.8	106.4
40%	60%	247.0	290.2	117.5
20%	80%	170.2	199.2	117.0
			Mean Recovery	**110.2%**



Intra Assay Precision

Three spiked samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated 11-ketotestosterone concentrations were:

Sample	11-Ketotestosterone Conc. (pg/mL)	%CV
1	349.5	4.6
2	262.3	6.8
3	184.0	6.6

Inter Assay Precision

Three spiked samples were diluted with Assay Buffer and run in duplicates in 19 assays run over multiple days by three operators. The mean and precision of the calculated 11-ketotestosterone concentrations were:

Sample	11-Ketoestosterone Conc. (pg/mL)	%CV
1	407.4	8.2
2	299.6	6.5
3	206.4	8.0

SAMPLE VALUES

Four urine samples from various species were tested in the assay. Adjusted neat concentrations of 11-ketotestosterone ranged from 1.162 to 6.576 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine Detection kit, K002-H1, the values ranged from 7.1 to 11.8 ng/mg creatinine.

Fecal samples from four different species were extracted and tested in the assay. Adjusted neat concentrations of 11-ketotestosterone ranged from 28.5 to 359.3 pg/mg dried feces.

Extracted serum from different species were also tested in the assay. Neat concentrations of 11-ketotestosterone in extracted human serum ranged from 144.5 to 449.3 pg/mL while extracted serum from White Strugeon fish ranged from 219.02 pg/mL to 28657.8 pg/mL.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
11-Ketotestosterone	100
Testosterone	2.03
11-Ketoandrostenedione	1.70
Dehydroandrosterone	1.48
DHEA	0.95
Progesterone	0.24
17-Hydroxyprogesterone	0.20
Epiandrosterone	0.12
Androsterone	0.08
17β-Estradiol	< 0.05

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

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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.

ASSAYS™
FOR SERIOUS SCIENCE

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