ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Androstenedione
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K070-H1
5 Plate Kit Catalog Number K070-H5

Species Independent

Sample Types Validated:

Saliva, Urine, 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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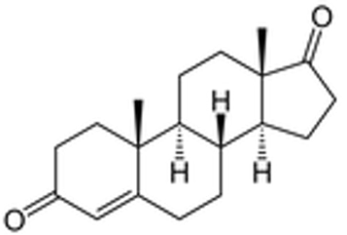
BACKGROUND

Androstenedione is a steroid hormone also known as androst-4-ene-3,17-dione. It is synthesized from dehydroepiandrosterone (DHEA) or 17-hydroxyprogesterone[1]. By itself, androstenedione is a weak androgen though it is a precursor for biosynthesis of stronger androgens, such as testosterone and estrogens. Therefore, androstenedione is classified as a controlled drug and a supplement to enhance athletic performance and body energy.



Androstenedione

In males, androgens are secreted by the testes and the adrenal cortex while, in females, they are secreted by the ovaries[2]. The production of androstenedione by adrenal glands is partially regulated by andrenocorticotripic hormone (ACTH). The amount of androstenedione in body fluids fluctuates throughout the day, with levels measuring highest in the morning and lowest in the evening[3]. The levels of androstenedione in serum also change with age and physiological conditions. During embryonic development, the levels increase with the highest values at birth and the levels then decrease during the first few years of development[4]. At puberty, the levels rise again for a few years, then taper down during menopause in females. Generally, a higher level of androstenedione may provide androgenic risk to females indicating higher androgenic activity, hirsutism or polycystic ovarian syndromes[4]. Due to androgenic and estrogenic function of this sex steroid, children and adolescents are highly sensitive to androstenedione exposure; thus, androstenedione is treated as a regulated substance by the DEA.

1. Yates, J., & Deshpande, N. (1974). Kinetic studies on the enzymes catalyzing the conversion of 17α-hydroxy-progesterone and dehydroepiandrosterone to androstenedione in the human adrenal gland in vitro. *Journal of Endocrinology, 6*(1), 27-35.
2. Möhle U., et al., (2002). Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male nonhuman primates. *General and Comparative Endocrinology, 129*(3), 135–45.
3. Weinstein, R. L., et al. (1974). Secretion of unconjugated androgens and estrogens by the normal and abnormal human testis before and after human chorionic gonadotropin. *Journal of Clinical Investigation, 53*(1), 1-6.
4. Goldman, J., et al. (1985). Contrast analysis for the evaluation of the circadian rhythms of plasma cortisol, androstenedione, and testosterone in normal men and the possible influence of meals. *The Journal of Clinical Endocrinology & Metabolism, 60*(1), 164-67.
5. Dorn, L. D., et al. (2009). Salivary gonadal and adrenal hormone differences in boys and girls with and without disruptive behavior disorders: Contextual variants. *Biological Psychology, 81*(1), 31-39.

ASSAY PRINCIPLE

The DetectX® Androstenedione Immunoassay Kit uses a specifically generated antibody to measure androstenedione in saliva or in extracted serum and plasma. This kit is not recommended for serum or plasma samples without extraction. The kit will also quantitatively measure androstenedione present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An androstenedione 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. An androstenedione-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 androstenedione to each well. After a two hour incubation the plate is washed and substrate added. The substrate reacts with the bound androstenedione-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. After making suitable correction for the dilution of the sample, the concentration of the androstenedione in the sample is calculated using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Urinary Creatinine Detection Kits	K002-H1/H5
17-Hydroxyprogesterone ELISA Kits	K053-H1/H5
Aldosterone ELISA & Chemiluminescent ELISA Kits	K052-H1/H5, -C1/C5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Dehydroepiandrosterone 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-3-Glucuronide (E1G) ELISA Kits	K036-H1/H5
Oxytocin ELISA & Chemiluminescent ELISA Kits	K048-H1/H5, -C1/C5
PGFM (13,14-Dihydro-15-keto-Prostaglandin $F_{1\alpha}$) ELISA Kits	K022-H1/H5
Pregnandiol-3-Glucuronide (PDG) ELISA Kits	K037-H1/H5
Progesterone ELISA Kits	K020-H1/H5
Progesterone Metabolites ELISA Kits	K068-H1/H5
Prolactin ELISA Kit	K040-H1
Testosterone ELISA Kits	K032-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
Clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.

Kit K070-H1 **or** -H5	1 **or** 5 Each	Catalog Number X061-1EA

Androstenedione Standard
Androrstenedione at 24 ng/mL in a special stabilizing solution.

Kit K070-H1 **or** -H5	125 µL **or** 625 µL	Catalog Number C254-125UL **or** -625UL

DetectX® Androstenedione Antibody
A color-coded rabbit polyclona antibody specific for Androstenedione.

Kit K070-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C253-3ML **or** -13ML

DetectX® Androstenedione Conjugate
A color-coded Androstenedione-peroxidase conjugate in a special stabilizing solution.

Kit K070-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C255-3ML **or** -13ML

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

Kit K070-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 K070-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K070-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K070-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K070-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.

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

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 µL.

A microplate shaker.

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 androstenedione standard used for this kit is an anabolic steroid and may have a number of known and unknown biological actions. Care must 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. If the desiccant is pink, discard the plate.

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 saliva samples and extracted serum and plasma samples. Samples containing visible particulate should be centrifuged prior to use. Androstenedione can be assayed in other 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.

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

SAMPLE PREPARATION

Serum and Plasma Samples

We have 3 detailed extraction options for liquid samples such as serum and plasma available on our website at www.arborassays.com/resources/#protocols. Please select the PDF entitled "Steroid Liquid Extraction Protocol". We recommend using diethyl ether or ethyl acetate with the protocols.

Saliva Samples

Saliva samples should be diluted at least 1:3 in diluted Assay Buffer prior to running in the assay. See our "Saliva Sample Handling Instructions" at www.arborassays.com/resources/#protocols.

Urine Samples

Urine samples should be diluted at least 1:1500 in diluted Assay Buffer prior to running in the assay. Most samples will require significant diluent to fall within the standard range.

Tissue Culture Media

For measuring tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM.

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

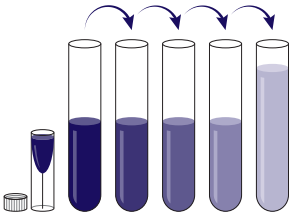
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 #6. Pipet 380 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #6. **Stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.** Carefully add 20 µL of the androstenedione stock solution to tube #1 and vortex completely. Take 150 µL of the androstenedione solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of androstenedione in tubes 1 through 6 will be 1,200, 400, 133.3, 44.4, 14.8, and 4.94 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**380**	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**20**	150	150	150	150	150
Final Conc (pg/mL)	1,200	400	133.3	44.4	14.8	4.94

Watch videos on sample preparation, useful tips and setting up an assay on our website at: http://www.arborassays.com/resources/#videos

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine androstenedione 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® Androstenedione Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Androstenedione 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 androstenedione 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. After subtracting the mean OD's for the NSB, create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader. 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://www.myassays.com/arbor-assays-androstenedione-enzyme-immunoassay-kit.assay

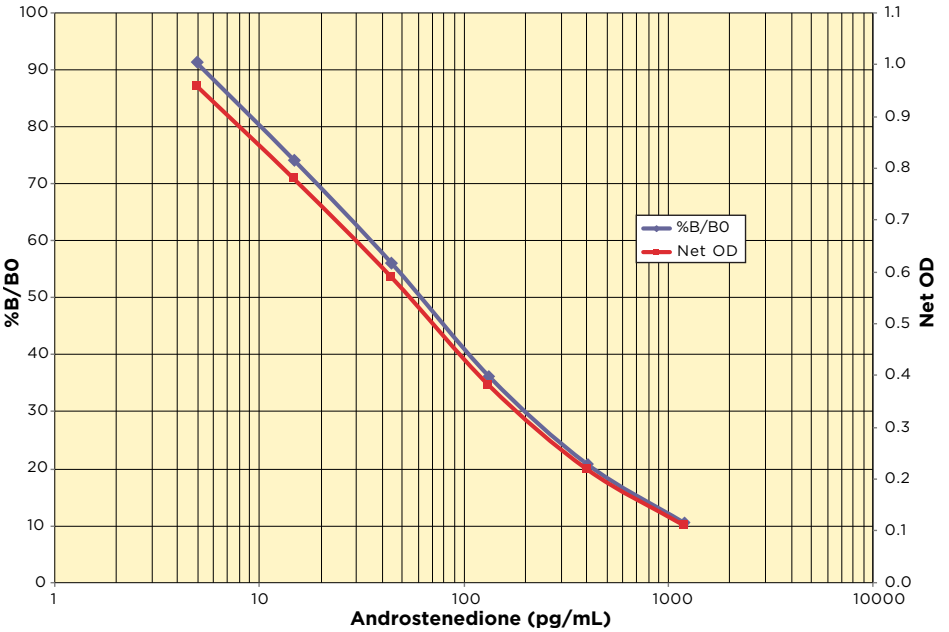
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Androstenedione Conc. (pg/mL)
NSB	0.075	0	-	-
Standard 1	0.185	0.110	10.5	1,200
Standard 2	0.294	0.219	20.8	400
Standard 3	0.455	0.380	36.2	133.3
Standard 4	0.664	0.589	56.0	44.4
Standard 5	0.854	0.779	74.1	14.8
Standard 6	1.034	0.959	91.2	4.94
B0	1.126	1.051	100	0
Sample 1	0.378	0.303	28.8	219.6
Sample 2	0.620	0.545	51.9	53.9

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

Conversion Factor: 100 pg/mL of androstenedione is equivalent to 349.1 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 #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 2.30 pg/mL.

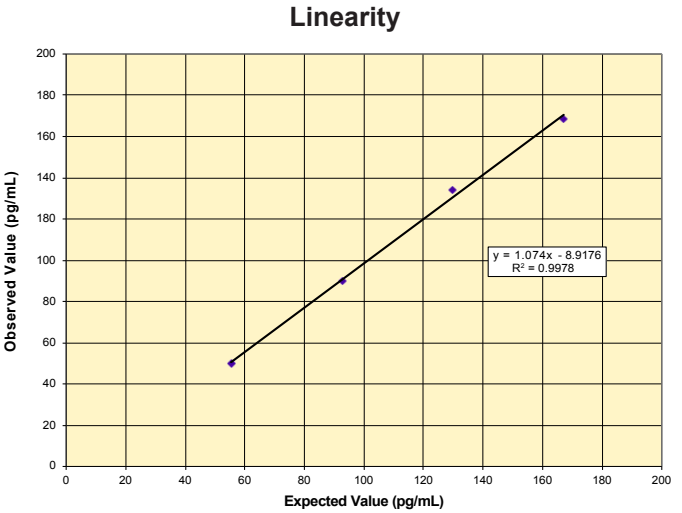
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration saliva sample.

Limit of Detection was determined as 1.48 pg/mL.

Linearity

Linearity was determined by taking two saliva samples diluted with Assay Buffer, one with a low androstenedione level and one with a higher level, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Saliva	Low Saliva	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	167.1	168.4	100.8
60%	40%	129.8	134.9	103.2
40%	60%	92.6	90.1	97.3
20%	80%	55.4	49.7	89.7
			Mean Recovery	**97.7%**



Intra Assay Precision

Four saliva samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated androstenedione concentrations were:

Sample	Androstenedione Conc. (pg/mL)	%CV
1	226.2	6.6
2	116.0	9.4
3	57.1	13.9
4	31.7	9.2

Inter Assay Precision

Four saliva samples were diluted with Assay Buffer and run in duplicates in 19 assays run over multiple days by four operators. The mean and precision of the calculated androstenedione concentrations were:

Sample	Androstenedione Conc. (pg/mL)	%CV
1	232.4	9.7
2	119.3	9.3
3	58.9	7.4
4	34.2	12.1

SAMPLE VALUES

Multiple serum and plasma samples were tested in the assay. Adjusted neat concentrations of androstenedione for the extracted samples ranged from 3,725 to 9,894 pg/mL, with an average of 5,785 pg/mL.

The Mayo Clinic Laboratories list normal serum levels of androstenedione for adult males as 400 to 1,500 pg/mL and for adult females as 300 to 2,000 pg/mL.

Eight saliva samples were tested in the assay. Adjusted neat concentrations of androstenedione for the samples ranged from 76.1 to 749.2 pg/mL, with an average of 421.0 pg/mL.

Four urine samples were tested in the assay and adjusted neat concentrations ranged rrom 81,525 pg/mL to 624,300 pg/mL, with an avereage of 262,046 pg/mL.

Each lab should estabilish its own range. This is to serve as a guide only.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Androstenedione	100%
Epiandrosterone	4.51%
Dehydroepiandrosterone (DHEA)	0.98%
Testosterone	0.48%
17-Hydroxyprogesterone	0.09%
Progesterone	0.03%
Estrone	0.0%
Estradiol	< 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 EIA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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