ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

EPIANDROSTERONE
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K063-H1
5 Plate Kit Catalog Number K063-H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts, Saliva, Urine, Extracted Serum/Plasma, and Tissue Culture Media

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

www.ArborAssays.com

WEB INSERT 190228

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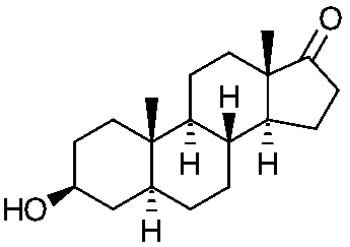
BACKGROUND

Epiandrosterone, or 3ß-androsterone, also known as 3ß-hydroxy-5α-androstan-17-one or 5α-androstan-3ß-ol-17-one, is a steroid hormone with weak androgenic activity. It is a natural metabolite of dehydroepiandrosterone (DHEA) via the 5α-reductase enzyme. It was first isolated in 1931, by Adolf Friedrich Johann Butenandt and Kurt Tscherning. They distilled over 17,000 litres of male urine, from which they got 50 milligrams of crystalline androsterone (most likely mixed isomers), which was sufficient to determine the chemical formula.



Epiandrosterone

Circulating steroid metabolites are extensively metabolised by liver and intestinal bacteria, generating a diverse range of fecal steroid metabolites. Steroid hormones, such as androgens, may be converted to polar water-soluble glucuronide and/or sulphate conjugates prior to excretion[1], which have been demonstrated by radiometabolism studies to occur in the feces of a taxonomically diverse range of mammalian species, e.g. primates[2], maned wolves[3], felids[4], and hyenas[5]. Enzyme immunoassays (EIA) that measure fecal testosterone metabolites are useful tools to monitor gonadal activity. Conjugated and free epiandrosterone has been shown to be part of the major androgens excreted in feces from hyenas[6].

1. Palme R,et al., “Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples.”, Ann. N Y Acad. Sci. 2005; 1040:162–71.
2. Möhle U,et al., “Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male nonhuman primates.”, Gen. Comp. Endocrinol. 2002; 129(3):135–45.
3. Velloso AL, Wasser SK, Monfort SL, Dietz JM. “Longitudinal fecal steroid excretion in maned wolves (*Chrysocyon brachyurus*)”., Gen. Comp. Endocrinol. 1998; 112(1):96–107.
4. Jewgenow K, Naidenko SV, Goeritz F, Vargas A, Dehnhard M. “Monitoring testicular activity of male Eurasian (Lynx lynx) and Iberian (*Lynx pardinus*) lynx by fecal testosterone metabolite measurement”., Gen. Comp. Endocrinol. 2006; 149(2):151–8.
5. Dloniak SM, French JA, Place NJ, Weldele ML, Glickman SE, Holekamp KE. “Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*)”., Gen. Comp. Endocrinol. 2004; 135(1):51–61.
6. Pribbenow S, East ML, Ganswindt A, Tordiffe ASW, Hofer H, Dehnhard M., “Measuring Faecal Epi-Androsterone as an Indicator of Gonadal Activity in Spotted Hyenas (Crocuta crocuta)”., 2015 ;PLoS ONE 10(6): e0128706.

ASSAY PRINCIPLE

The DetectX® Epiandrosterone Immunoassay kit uses a specifically generated antibody to measure Epiandrosterone in urine, saliva, and fecal samples, or in extracted serum and plasma. This kit is not recommended for serum or plasma samples without extraction. The kit will also quantitatively measure epiandrosterone present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An epiandrosterone 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 Epiandrosterone-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 epiandrosterone to each well. After a two hour incubation the plate is washed and substrate added. The substrate reacts with the bound epiandrosterone-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 epiandrosterone 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.
Urinary Creatinine Detection Kits	K002-H1/H5
17-Hydroxyprogesterone EIA kits	K053-H1/H5
Aldosterone EIA & CLIA Kits	K052-H1/H5, -C1/C5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Dehydroepiandrosterone Sulfate (DHEA-S) EIA Kits	K054-H1/H5
Estradiol Non-Invasive & Serum EIA Kits	K030-H1/H5, KB30-H1/H5
Estrone-3-Glucuronide (E1G) EIA Kits	K036-H1/H5
Oxytocin EIA & CLIA Kits	K048-H1/H5, -C1/C5
PGFM (13,14-Dihydro-15-keto-Prostaglandin $F_{1\alpha}$) EIA Kits	K022-H1/H5
Pregnandiol-3-Glucuronide (PDG) EIA Kits	K037-H1/H5
Progesterone EIA Kits	K025-H1/H5
Prolactin EIA Kit	K040-H1
Testosterone EIA Kits	K032-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plate
Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.
Kit K063-H1 **or** -H5 1 **or** 5 Each — Catalog Number X016-1EA

Epiandrosterone Standard
Epiandrosterone at 1,000 ng/mL in a special stabilizing solution.
Kit K063-H1 **or** -H5 125 µL **or** 625 µL — Catalog Number C233-125UL **or** -625UL

DetectX® Epiandrosterone Antibody
A color-coded rabbit polyclonal antibody specific for Epiandrosterone.
Kit K063-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C231-3ML **or** -13ML

DetectX® Epiandrosterone Conjugate
A color-coded Epiandrosterone-peroxidase conjugate in a special stabilizing solution.
Kit K063-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C232-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that must be diluted with deionized or distilled water.
Kit K063-H1 **or** -H5 28 mL **or** 55 mL — Catalog Number X067-28ML **or** -55ML

Wash Buffer Concentrate
A 20X concentrate that must be diluted with deionized or distilled water.
Kit K063-H1 **or** -H5 30 mL **or** 125 mL — Catalog Number X007-30ML **or** -125ML

TMB Substrate
Kit K063-H1 **or** -H5 11 mL **or** 55 mL — Catalog Number X019-11ML **or** -55ML

Stop Solution
A 1M solution of hydrochloric acid. **CAUSTIC**.
Kit K063-H1 **or** -H5 5 mL **or** 25 mL — Catalog Number X020-5ML **or** -25ML

Plate Sealer
Kit K063-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.

Ethanol or methanol will be needed for extraction of fecal 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 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 urine and saliva samples, extracted serum and plasma samples, dried fecal extracts and tissue culture media. Samples containing visible particulate should be centrifuged prior to use. Epiandrosterone 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.

Epiandrosterone is identical across all species and we expect this kit to measure Epiandrosterone from all sources. The end user should evaluate recoveries of Epiandrosterone 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.

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 1:5 in diluted 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.

Saliva Samples

Saliva samples should be diluted at least 1:4 in diluted Assay Buffer prior running in the assay. See our Saliva Sample Handling Instructions at www.arborassays.com/resources/#protocols.

Tissue Culture Media

For measuring Epiandrosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

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

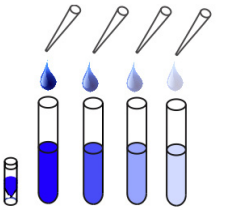
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 360 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #6. **The Epiandrosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 40 µL of the Epiandrosterone stock solution to tube #1 and vortex completely. Take 100 µL of the Epiandrosterone 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 Epiandrosterone in tubes 1 through 6 will be 100, 25, 6.25, 1.563, 0.391 and 0.098 ng/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**360**	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**40**	100	100	100	100	100
Final Conc (ng/mL)	100	25	6.25	1.563	0.391	0.098

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 Epiandrosterone 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® Epiandrosterone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Epiandrosterone 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. 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 Epiandrosterone 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://www.myassays.com/arbor-assays-epiandrosterone-eia-kit.assay

MyAssays

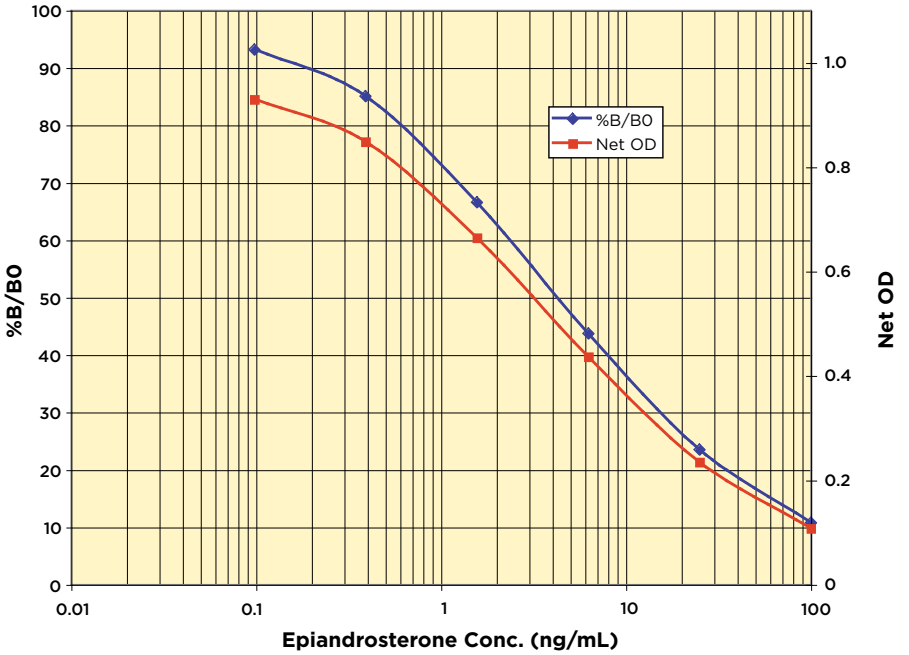
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Epiandrosterone Conc. (ng/mL)
NSB	0.081	0	-	-
Standard 1	0.189	0.108	10.8	100
Standard 2	0.316	0.235	23.5	25
Standard 3	0.518	0.437	43.8	6.25
Standard 4	0.746	0.665	66.6	1.563
Standard 5	0.930	0.849	85.1	0.391
Standard 6	1.011	0.930	93.2	0.098
B0	1.079	0.998	100	0
Sample 1	0.495	0.414	41.5	7.16
Sample 2	0.746	0.665	66.6	1.62

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

Conversion Factor: 100 ng/mL of Epiandrosterone is equivalent to 344.3 nM.

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 0.120 ng/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 human urine sample.

Limit of Detection was determined as 0.107 ng/mL.

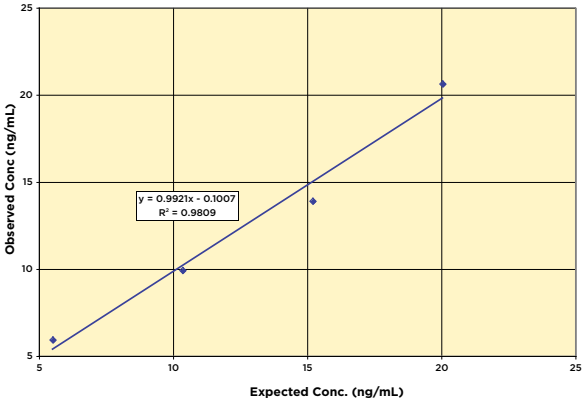
Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low Epiandrosterone level of 0.675 ng/mL and one with a higher level of 24.9 ng/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used. Linearity in fecal extracts was determined in a similar manner.

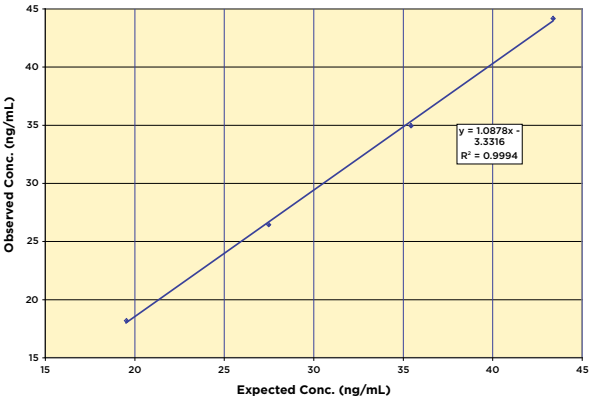
High Urine	Low Urine	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	20.06	20.62	102.8
60%	40%	15.21	13.89	91.3
40%	60%	10.37	9.92	95.7
20%	80%	5.52	5.92	107.2
			Mean Recovery	**99.3%**

High Fecal Sample	Low Fecal Sample	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	43.40	44.14	101.7
60%	40%	35.45	34.93	98.5
40%	60%	27.50	26.42	96.0
20%	80%	19.55	18.15	92.8
			Mean Recovery	**97.3%**

Urine Linearity



Fecal Extract Linearity



Intra Assay Precision

Five human urine samples were diluted with Assay Buffer and run in replicates of ≥ 19 in an assay. The mean and precision of the calculated epiandrosterone concentrations were:

Sample	Epiandrosterone Conc. (ng/mL)	%CV
1	13.84	6.4
2	7.81	9.5
3	5.45	7.9
4	2.45	9.6
5	2.08	8.1

Inter Assay Precision

Five human urine samples were diluted with Assay Buffer and run in duplicates in ≥ 21 assays run over multiple days by four operators. The mean and precision of the calculated epiandrosterone concentrations were:

Sample	Epiandrosterone Conc. (ng/mL)	%CV
1	13.84	10.1
2	8.17	16.2
3	5.34	14.8
4	2.25	18.8
5	1.97	19.8

SAMPLE VALUES

Multiple human serum and plasma samples were tested in the assay. Adjusted neat concentrations of epiandrosterone for the extracted samples ranged from 33.5 to 55.6 ng/mL with an average 41 ng/mL.

Human urine samples were tested in the assay. Adjusted neat concentrations of epiandrosterone for the urine samples ranged from 121.5 to 1,131 ng/mL with an average of 511.1 ng/mL.

Extracted fecal samples from a male fennec fox read between 25.1 and 1,027 ng/mL. **Fennec fox samples were the kindly supplied by Rachel Santymire, Linclon Park Zoo, Chicago, IL.**

For urine samples we recommend the use of a kit to measure creatinine to normalize for urine output such as the DetectX® Urinary Creatinine detection kit, K002-H1 and K002-H5.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Androstenedione	161.10
Epiandrosterone glucuronide	112.5
Androsterone	36.5
Dehydroeipandrosterone (DHEA)	33.8
Epiandrosterone sulphate	32.4
Androsterone sulphate	11.8
DHEA sulphate	11.8
Andrenosterone	4.54
19-Nortestosterone	2.3
Progesterone	2.1
DHT	1.9
Testosterone	1.8
Estrone	0.75
17b-Estradiol	0.14
Cortisol	0.12
Corticosterone	0.1

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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