ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Testosterone
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K032-H1
5 Plate Kit Catalog Number K032-H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts, 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

K032-H WEB 190910

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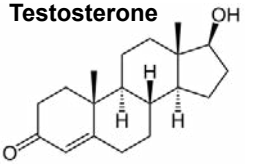
BACKGROUND

Testosterone, $C_{19}H_{28}O_2$, (4-Androsten-17β-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 hair[3]. In the absence of testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage. In addition, testosterone is essential for health and well-being[4] as well as the prevention of osteoporosis[5]. 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 hormone[6]. Testosterone plays a significant role in glucose homeostasis and lipid metabolism. The metabolic syndrome is a clustering of risk factors predisposing to type 2 *diabetes mellitus*, atherosclerosis and cardiovascular morbidity and mortality. 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.

Testosterone is observed in most vertebrates. Fish make a slightly different form called 11-ketotestosterone[7]. Its counterpart in insects is ecdysone[8] These ubiquitous steroids suggest that sex hormones have an ancient evolutionary history[9].

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2. Reed WL, et. al., “Physiological effects on demography: a long-term experimental study of testosterone’s effects on fitness”. 2006, Am. Nat. 167:667–83.
3. Mooradian AD, Morley JE, Korenman SG., “Biological actions of androgens”. 1987, Endocr. Rev. 8:1–28.
4. Bassil N, Alkaade S, Morley JE., “The benefits and risks of testosterone replacement therapy: a review”. 2009, Ther. Clin. Risk Manag. 5:427–48.5.
5. Tuck SP, Francis RM., “Testosterone, bone and osteoporosis”. 2009, Frnt. Horm. Res. 37:123–32.
6. Dabbs M, Dabbs JM., In: “Heroes, rogues, and lovers: testosterone and behavior.” 2000, New York: McGraw-Hill.
7. Nelson, RF., In: “An introduction to behavioral endocrinology.” 2005, Sunderland, Mass: Sinauer Associates. pp. 143.
8. De Loof A., “Ecdysteroids: the overlooked sex steroids of insects? Males: the black box”. 2006, Insect Sci., 13:325–338.
9. Mechoulam R, Brueggemeier RW, Denlinger DL, R.; Brueggemeier, R. W.; Denlinger, D. L., “Estrogens in insects”., 1984, J. Cell. and Mol. Life Sci., 40:942–944.

ASSAY PRINCIPLE

The DetectX® Testosterone Immunoassay Kit uses a specifically generated antibody to measure testosterone and its metabolites in urine and fecal samples, or in extracted serum and plasma. This kit is not recommended for serum, plasma, or saliva samples without extraction. The kit will quantitatively measure Testosterone present in reconstituted buffer samples and tissue culture media samples. 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 rabbit 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 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 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.
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
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 K032-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

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

Kit K032-H1 **or** -H5	70 µL **or** 350 µL	Catalog Number C113-70UL **or** -350UL

DetectX® Testosterone Antibody
A rabbit polyclonal antibody specific for testosterone

Kit K032-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C111-3ML **or** -13ML

DetectX® Testosterone Conjugate
A testosterone-peroxidase conjugate in a special stabilizing solution.

Kit K032-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 K032-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 K032-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K032-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K032-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K032-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 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 dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Testosterone 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

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

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 1:4 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.

Tissue Culture Media

For measuring testosterone 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

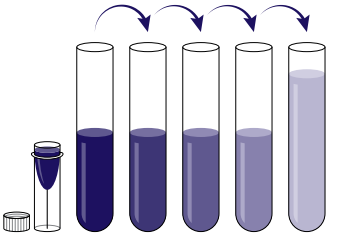
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 #7. Pipet 475 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #7. **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 tube #1 and vortex completely. Take 200 µL of the testosterone 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 testosterone in tubes 1 through 7 will be 10,000, 4,000, 1,600, 640, 256, 102.4 and 40.96 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)	**475**	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**25**	200	200	200	200	200	200
Final Conc (pg/mL)	10,000	4,000	1,600	640	256	102.4	40.96

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 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® 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. 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.

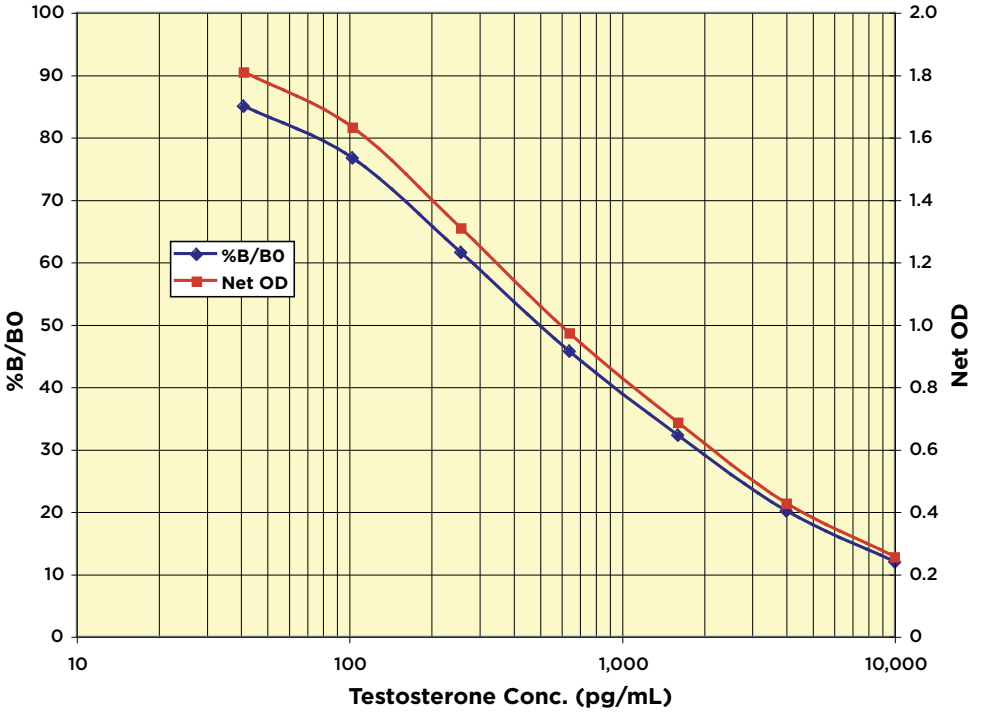
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-testostrone-eia-kit.assay

TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Testosterone Conc. (pg/mL)
NSB	0.044	0	-	-
Standard 1	0.299	0.255	12.0	10,000
Standard 2	0.472	0.428	20.1	4,000
Standard 3	0.731	0.687	32.3	1,600
Standard 4	1.017	0.973	45.7	640
Standard 5	1.354	1.310	61.6	256
Standard 6	1.676	1.632	76.7	102.4
Standard 7	1.853	1.809	85.0	40.96
B0	2.172	2.128	100	0
Sample 1	0.751	0.707	33.2	1,434.0
Sample 2	1.327	1.283	60.3	284.4

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

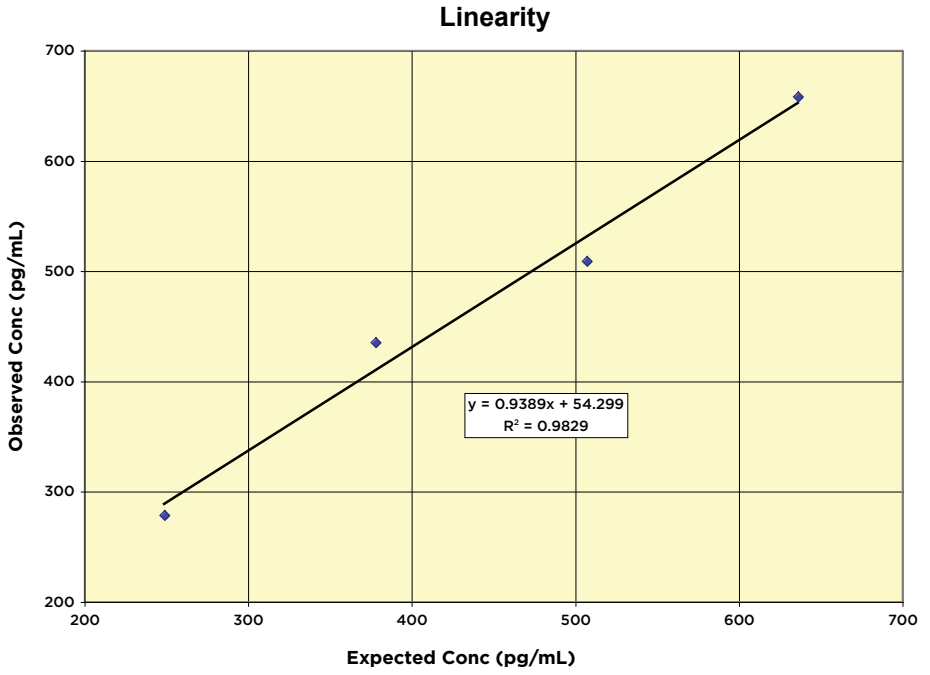
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 9.92 pg/mL.**

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 human urine sample. **Limit of Detection was determined as 30.6 pg/mL.**

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted testosterone level of 119.9 pg/mL and one with a higher diluted level of 765.6 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%	636.4	657.9	103.4
60%	40%	507.3	508.6	100.3
40%	60%	378.2	435.1	115.1
20%	80%	249.0	278.3	111.7
			Mean Recovery	**107.6%**



Intra Assay Precision

Three human samples were diluted with 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	1,384	5.7
2	275.8	11.1
3	147.5	15.8

Inter Assay Precision

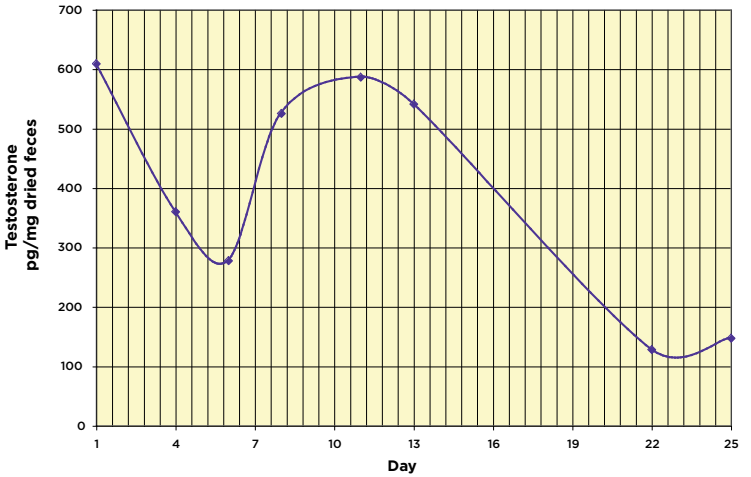
Three human samples were diluted with Assay Buffer and run in duplicates in fifteen assays run over multiple days by three operators. The mean and precision of the calculated Testosterone concentrations were:

Sample	Testosterone Conc. (pg/mL)	%CV
1	1,436	6.2
2	283.4	11.2
3	150.3	10.5

SAMPLE VALUES

Eleven urine samples from various species were tested in the assay. Adjusted neat concentrations of Testosterone ranged from 2.331 to 215.0 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine Detection kit, K002-H1, the values ranged from 16.2 to 1,567 ng/mg creatinine.

Fecal samples from Dune, a male fennec fox, were extracted and tested in the assay.



The fennec fox sample was the kind gift from Jocelyn Bryant, Brookfield Zoo, Brookfield, IL.

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	100%	17β-Estradiol	0.02%
5α-Dihydrotestosterone	56.8%	Progesterone	< 0.02%
Androstendione	0.27%	Pregnenolone	< 0.02%
Androsterone	0.04%	Hydrocortisone	< 0.02%
DHEA	0.04%	Cholic Acid	< 0.02%
Cholesterol	0.03%	Cholic Derivatives	< 0.02%

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.

ASSAYS™
FOR SERIOUS SCIENCE

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