ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

17-Hydroxyprogesterone
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K053-H1
5 Plate Kit Catalog Number K053-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

K053-H WEB 210302

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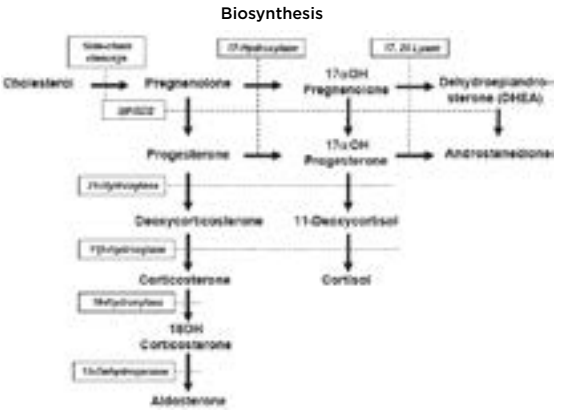
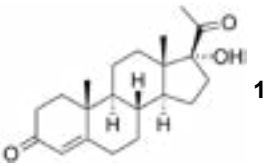
BACKGROUND

17-Hydroxyprogesterone, $C_{21}H_{30}O_3$, (4-pregen-17-ol-3, 20-dione, 17HO-P, OHPG) is a steroid hormone from the androgen group and is found in mammals, reptiles, birds, and other vertebrates. It was first isolated from the adrenal glands of cattle by Pfiffner and North at Park, Davis and Company in Detroit, Michigan in 1940[1]. It is derived from progesterone via 17-hydroxylase, a P450c17 enzyme, or from 17-hydroxypregnenolone via 3ß-hydroxysteroid dehydrogenase 2/Δ^{5-4} isomerase. It is primarily produced in the adrenal glands and to some degree in the gonads, specifically the corpus luteum of the ovary.



The adrenal glands, ovaries, testes, and placenta produce 17-hydroxyprogesterone. It is hydroxylated at the 11 and 21 position to produce cortisol. Deficiency of either 11- or 21-hydroxylase results in decreased cortisol synthesis, and feedback inhibition of adrenocorticotropic hormone (ACTH) secretion is lost. Consequently, increased pituitary release of ACTH will increase production of 17HO-P. If 17α-hydroxylase (which allows formation of 17HO-P from progesterone) or 3ß-hydroxysteroid dehydrogenase type 2 (which allows formation of 17-hydroxyprogesterone formation from 17-hydroxypregnenolone) are deficient, 17HO-P levels are low with possible increase in progesterone or pregnenolone respectively. Normal levels are 3-90 ng/dL in children, and in women, 20-100 ng/dL prior to ovulation, and 100-500 ng/dL during the luteal phase[2,3].



17-Hydroxyprogesterone

Congenital adrenal hyperplasia (CAH) is caused by inherited defects in steroid biosynthesis. The resulting hormone imbalances with reduced glucocorticoids and mineralocorticoids and elevated 17HO-P and androgens can lead to life-threatening, salt-wasting crisis in the newborn and incorrect gender assignment of virtualized females. Adult-onset CAH may result in hirsutism or infertility in females.

1. Pfiffner JJ., and North HB., "17-ß-Hydroxyprogesterone", J.Biol.Chem., 1940, 132:459-460.
2. Abbassi-Ghanavati M., Greer LG., Cunningham FG., "Pregnancy and laboratory studies: a reference table for clinicians.", Obstet Gynecol., 2009, 114:1326-1331.
3. Kratz A., Ferraro M., Sluss PM., Lewandrowski KB., "Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values.", N. Engl. J. Med., 2004, 351:1548-1563.

ASSAY PRINCIPLE

The DetectX® 17-Hydroxyprogesterone Immunoassay kit uses a specifically generated antibody to measure 17-Hydroxyprogesterone (17HO-P) and its metabolites in urine and fecal samples, or in extracted serum and plasma. This kit is not recommended for serum or plasma samples without extraction. The kit will quantitatively measure 17HO-P present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. A 17HO-P 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 17HO-P-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 17HO-P to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound 17HO-P-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 17HO-P 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.
Cortisol EIA Kits	K003-H1/H5, K003-H1W/H5W
Corticosterone EIA & CLIA Kits	K014-H1/H5, K014-C1/C5
Cortisone EIA & CLIA Kits	K017-H1/H5, K017-C1/C5
Estradiol Non-Invasive & Serum EIA Kits	K030-H1/H5, KB30-H1/H5
Estrone EIA Kits	K031-H1/H5
Progesterone EIA Kits	K025-H1/H5
PGFM (13,14,Dihydro-15-keto-PGFa) EIA Kits	K022-H1/H5
Testosterone EIA Kits	K032-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

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

Kit K053-H1 **or** -H5 1 **or** 5 Each — Catalog Number X061-1EA

17-Hydroxyprogesterone Standard
17-Hydroxyprogesterone at 120,000 pg/mL in a special stabilizing solution.

Kit K053-H1 **or** -H5 70 µL **or** 350 µL — Catalog Number C192-70UL **or** -350UL

DetectX® 17-Hydroxyprogesterone Antibody
A color-coded sheep polyclonal antibody specific for 17-Hydroxyprogesterone.

Kit K053-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C190-3ML **or** -13ML

DetectX® 17-Hydroxyprogesterone Conjugate
A color-coded 17-Hydroxyprogesterone-peroxidase conjugate in a special stabilizing solution.

Kit K053-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C191-3ML **or** -13ML

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

Kit K053-H1 **or** -H5 28 mL **or** 55 mL — Catalog Number X065-28ML **or** -55ML

Wash Buffer Concentrate
A 20X concentrate that must be diluted with deionized or distilled water.

Kit K053-H1 **or** -H5 30 mL **or** 125 mL — Catalog Number X007-30ML **or** -125ML

TMB Substrate
Kit K053-H1 **or** -H5 11 mL **or** 55 mL — Catalog Number X019-11ML **or** -55ML

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

Kit K053-H1 **or** -H5 5 mL **or** 25 mL — Catalog Number X020-5ML **or** -25ML

Plate Sealer
Kit K053-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 µL, 50 µL, and 100 µL.

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 extracted serum and plasma samples, non-extracted urine, tissue culture samples and dried fecal extracts. Samples containing visible particulate should be centrifuged prior to using. 17HO-P 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.

17HO-P is identical across all species and we expect this kit to measure 17HO-P from all sources. The end user should evaluate recoveries of 17HO-P 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 or ethyl acetate to serum or plasma samples at a 5:1 (v/v) solvent:sample ratio.
2. Mix solutions by vortexing for 2 minutes. Allow layers to separate for 5 minutes.
3. Freeze samples in a dry ice/ethanol bath and pipet off the solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining the solvent solutions.
4. Dry pooled solvent extracts 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 diluted Assay Buffer. A minimum of 125 µL of 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 < 2.5%.

Urine Samples

Urine samples should be diluted at least 1:2 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.

Tissue Culture Media

For measuring 17HO-P 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

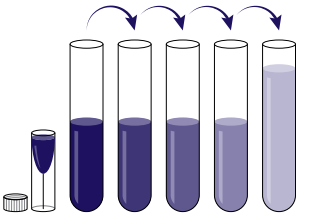
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 380 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #6. **The 17-Hydroxyprogesterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 20 µL of the 17-Hydroxyprogesterone stock solution to tube #1 and vortex completely. Take 100 µL of the 17HO-P 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 17-Hydroxyprogesterone in tubes 1 through 6 will be 6,000, 2,000, 666.7, 222.2, 74.07 and 24.69 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**	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**20**	100	100	100	100	100
Final Conc (pg/mL)	6,000	2,000	666.7	222.2	74.07	24.69

Watch videos on sample preparation and setting up an assay on our website at:
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 17-Hydroxyprogesterone 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® 17-Hydroxyprogesterone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® 17-Hydroxyprogesterone 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 1 hour. 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 17-Hydroxyprogesterone 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-17-hydroxyprogesterone-eia-kit.assay

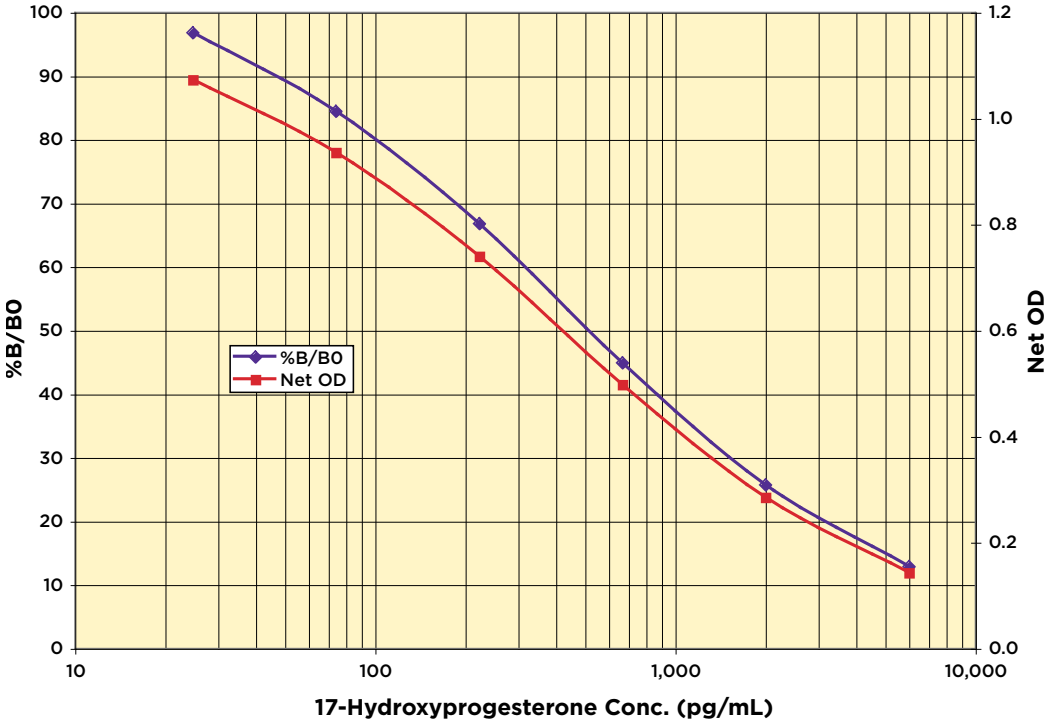
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	17-Hydroxyprogesterone Conc. (pg/mL)
NSB	0.062	0	-	-
Standard 1	0.205	0.143	13.0	6,000
Standard 2	0.347	0.285	25.7	2,000
Standard 3	0.560	0.498	44.9	666.7
Standard 4	0.802	0.740	66.8	222.2
Standard 5	0.998	0.936	84.5	74.07
Standard 6	1.135	1.073	96.8	24.69
B0	1.170	1.108	100	0
Sample 1	0.426	0.364	32.8	1,293.4
Sample 2	0.659	0.597	53.9	425.5

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

Conversion Factor: 100 pg/mL of 17-Hydroxyprogesterone is equivalent to 302.6 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 20.3 pg/mL.

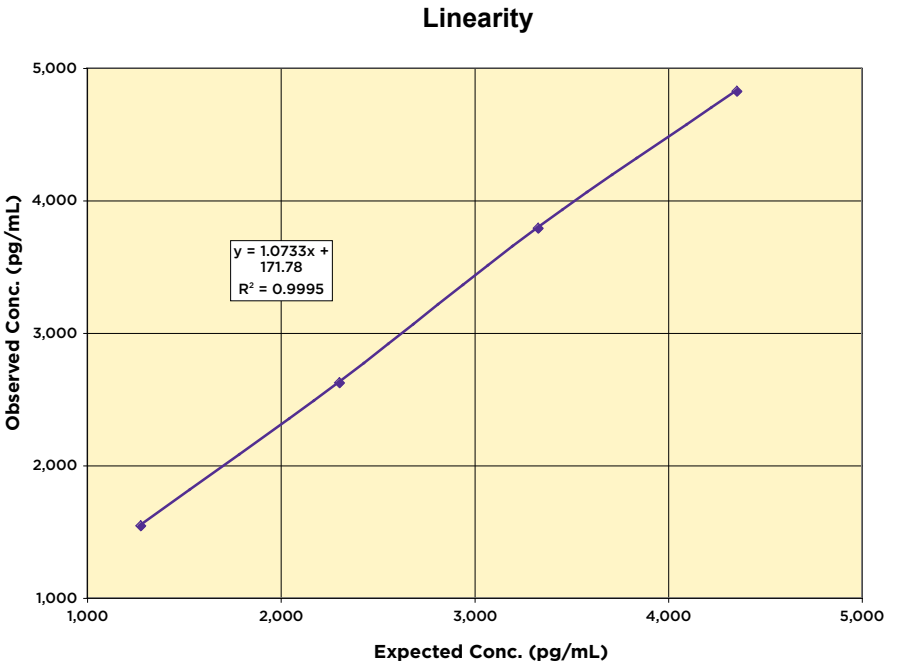
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human urine sample.

Limit of Detection was determined as 15.4 pg/mL

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted 17-hydroxyprogesterone level of 252.1 pg/mL and one with a higher diluted level of 5,381 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%	4,355	4,824	110.8
60%	40%	3,330	3,789	113.8
40%	60%	2,304	2,623	113.8
20%	80%	1,278	1,543	120.8
			Mean Recovery	**114.8%**



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 17-Hydroxyprogesterone concentrations were:

Sample	17-Hydroxyprogesterone Conc. (pg/mL)	%CV
1	1,265.7	5.4
2	450.0	6.5
3	168.5	7.9

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in eighteen assays run over multiple days by four operators. The mean and precision of the calculated 17-Hydroxyprogesterone concentrations were:

Sample	17-Hydroxyprogesterone Conc. (pg/mL)	%CV
1	1,204.8	7.0
2	444.1	6.5
3	162.9	10.6

SAMPLE VALUES

Multiple human serum samples were tested in the assay. Adjusted neat concentrations of 17-Hydroxyprogesterone for the extracted male and non-pregnant female samples ranged from 2,220 to 406 pg/mL with an average 1,861 pg/mL.

Multiple human EDTA plasma samples were tested in the assay. Adjusted neat concentrations of 17-Hydroxyprogesterone for the extracted male and non-pregnant female samples ranged from 2,077 to 457 pg/mL with an average 787 pg/mL.

Human serum samples from pregnant females were tested in the assay. Adjusted neat concentrations of 17-Hydroxyprogesterone for the extracted samples ranged from 5,791 to 3,642 pg/mL.

Human urine samples were tested in the assay. Adjusted neat concentrations of 17-Hydroxyprogesterone for the urine samples from non-pregnant females ranged from 15,067 to 2,492 pg/mL with an average of 6,425 pg/mL. Two urine samples from a pregnant female read 60,662 and 53,813 pg/mL.

Extracted fecal samples from a pregnant Iberian lynx read between 114.8 and 1,169 ng/mL over the gestation period. **Iberian lynx samples were from Martin Dehnhard, Leibniz Institute for Zoo & Wildlife Research, Berlin.**

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 (%)
17-Hydroxyprogesterone	100%
17a-Hydroxypregnanolone	17.4%
Progesterone	0.29
11a-Hydroxyprogesterone	0.08
5a-dihydroprogesterone	0.04
20a-Hydroxyprogesterone	< 0.01
Androstendione	< 0.01
Cholesterol	< 0.01
Corticosterone	< 0.01
Cortisol	< 0.01
Pregnenolone	< 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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