

ARBOR ASSAYS™
Interactive Assay Solutions™



DetectX[®]

Thyroxine (T₄) Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K050-H1

5 Plate Kit Catalog Number K050-H5

Species Independent

Multi-Format Kit for Multiple Sample Types

Sample Types Validated:

**Serum, EDTA and Heparin Plasma,
Urine, TCM and Fecal Extracts**

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

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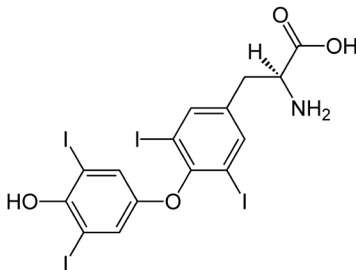
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BACKGROUND

Thyroxine is the main hormone produced by the thyroid gland. The thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. Iodine is necessary for the production of T_3 and T_4 . A deficiency of iodine leads to decreased production of T_3 and T_4 , enlarges the thyroid tissue and will cause the disease known as goitre. The major form of thyroid hormone in the blood is thyroxine (T_4), which has a longer half-life than T_3 . The ratio of T_4 to T_3 released into the blood is roughly 20 to 1. T_4 is converted to the active T_3 (three to four times more potent than T_4) within cells by deiodinases (5'-iodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T_{1a}) and thyronamine (T_{0a}). All three isoforms of the deiodinases are selenium-containing enzymes, thus dietary selenium is essential for T_3 production. Hypothyroidism is the condition that results from under-production of thyroxine by the thyroid gland either because the gland is naturally underactive or because radioiodine therapy or surgery for an overactive gland has resulted in underactivity. Thyroxine is taken to replace the deficiency which exists in such situations and therefore to restore normal metabolic activity. Thyroid hormone production is regulated via pituitary thyrotropin (TSH) modulation of thyroxine (T_4) prohormone secretion by the thyroid gland and regulation of active triiodothyronine (T_3) production in peripheral tissues via metabolic events influencing enzyme.



ASSAY PRINCIPLE

The DetectX® Thyroxine (T₄) Multi-Format Immunoassay kit is designed to quantitatively measure T₄ present in serum, plasma, urine, extracted dried fecal samples, and tissue culture media samples. Please read the complete kit insert before performing this assay. This kit measures total T₄ in serum and plasma and in extracted fecal samples.

The kit offers 2 standard curve ranges. For serum and plasma samples we recommend using 10 µL of standards or samples. The assay concentration range for T₄ will be from 20 ng/mL to 0.625 ng/mL. For urine samples we recommend alternatively using 100 µL of standards or samples. Assay concentrations of T₄ that range from 2 ng/mL to 0.0625 ng/mL can be measured.

A T₄ stock solution is provided to generate standard curves 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 mouse antibodies. A T₄-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to T₄ to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound T₄-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 T₄ 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 (2 or 10 Plates)	K002-H1/H5
Cortisol Enzyme Immunoassay Kits (Strip Wells)	K003-H1/H5
Cortisol Enzyme Immunoassay Kits (Whole Plate)	K003-H1W/H5W
Glucose Colorimetric Detection Kit (2 Plate)	K039-H1
Glucose Fluorescent Detection Kit (2 Plate)	K039-F1
Triiodothyronine (T ₃) Enzyme Immunoassay Kits	K056-H1/H5



SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) coated with goat anti-mouse IgG.

Kit K050-H1 or -H5 1 or 5 Each Catalog Number X012-1EA

Thyroxine (T₄) Standard

Thyroxine at 1,000 ng/mL in a special stabilizing solution.

Kit K050-H1 or -H5 40 µL or 200 µL Catalog Number C177-40UL or -200UL

DetectX[®] Thyroxine (T₄) Antibody

A mouse monoclonal antibody specific for thyroxine

Kit K050-H1 or -H5 3 mL or 13 mL Catalog Number C175-3ML or -13ML

DetectX[®] Thyroxine (T₄) Conjugate

A thyroxine-peroxidase conjugate in a special stabilizing solution.

Kit K050-H1 or -H5 3 mL or 13 mL Catalog Number C176-3ML or -13ML

Assay Buffer Concentrate

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

Kit K050-H1 or -H5 28 mL or 55 mL Catalog Number X065-28ML or -55ML

Dissociation Reagent

Kit K050-H1 or -H5 1 mL or 5 mL Catalog Number X058-1ML or -5ML

Dissociation Reagent is to be used only with Serum and Plasma samples.

Wash Buffer Concentrate

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

Kit K050-H1 or -H5 30 mL or 125 mL Catalog Number X007-30ML or -125ML

TMB Substrate

Kit K050-H1 or -H5 11 mL or 55 mL Catalog Number X019-11ML or -55ML

Stop Solution

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

Kit K050-H1 or -H5 5 mL or 25 mL Catalog Number X020-5ML or -25ML

Plate Sealer

Kit K050-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.

Repeater pipet with disposable tips capable of dispensing 25 and 100 μ L.

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.

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.



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SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma, urine and for tissue culture samples. It has also been validated for dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit. Thyroxine can be assayed in other sample types by using one of the extraction protocols available on our website at: www.arborassays.com/resources/#protocols

Thyroxine is identical across all species and we expect this kit may measure thyroxine from sources other than human. **The end user should evaluate recoveries of thyroxine in other samples being tested.**

SAMPLE PREPARATION

Serum and Plasma Samples

10 μ L Format (See page 9) Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total thyroxine concentration in serum or plasma. **Dissociation Reagent is to be used only with Serum and Plasma samples.** Allow the Dissociation Reagent to warm completely to **room temperature** before use.

We suggest pipetting 5 μ L of Dissociation Reagent into 1 mL Eppendorf tubes. Add 5 μ L of serum or plasma to the Dissociation Reagent in the tube, vortex gently and incubate at room temperature for 5 minutes or longer. Dilute with 90 μ L of supplied Assay Buffer. This 1:20 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be \geq 1:20.

Urine Samples

100 μ L Format (See page 9) 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 2 and 10 plate Urinary Creatinine Detection kits, K002-H1 and K002-H5.

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%.

Tissue Culture Media

For measuring thyroxine 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, or stored at \leq -20°C until assaying.

REAGENT PREPARATION

Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

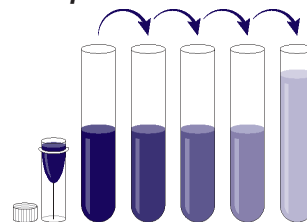
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 - 10 µL Assay Format for Serum and Plasma Samples

Label test tubes as #1 through #6. Pipet 490 µL of Assay Buffer into tube #1 and 100 µL into tubes #2 to #6. **The thyroxine stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 µL of the thyroxine stock solution to tube #1 and vortex completely. Take 100 µL of the thyroxine 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 thyroxine in tubes 1 through 6 will be 20, 10, 5, 2.5, 1.25, and 0.625 ng/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	490	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	10	100	100	100	100	100
Final Conc (ng/mL)	20	10	5	2.5	1.25	0.625

Standard Preparation - 100 µL Assay Format for Urine Samples

Label one test tube as Stock 2 and six as #1 through #6. Pipet 996 µL of Assay Buffer into the Stock 2 tube and 300 µL into tubes #1 to #6. **The thyroxine stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 4 µL of the thyroxine stock solution the Stock 2 tube and vortex completely. Take 300 µL of the thyroxine solution in the Stock 2 tube, add it to tube #1 and vortex completely. Repeat the serial dilutions for tubes #2 through #6. The concentration of thyroxine in tubes 1 through 6 will be 2,000, 1,000, 500, 250, 125, and 62.5 pg/mL.

	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	996	300	300	300	300	300	300
Addition	Stock	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	4	300	300	300	300	300	300
Final Conc (pg/mL)	4,000	2,000	1,000	500	250	125	62.5

Use all Standards within 2 hour of preparation.



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ASSAY PROTOCOL - 10 μ L AND 100 μ L

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine thyroxine 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 10 μ L (100 μ L for alternate format) of samples or standards into wells in the plate.
3. Pipet 35 μ L (125 μ L for alternate format) of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 10 μ L (100 μ L for alternate format) of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 μ L of the DetectX® Thyroxine Conjugate to each well using a repeater pipet.
6. Add 25 μ L of the DetectX® Thyroxine 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. If the plate is not shaken signals bound will be approximately 40% lower.
8. Aspirate the plate and wash each well 4 times with 300 μ L wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 μ L 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 μ L of the Stop Solution to each well, using a repeater or a multichannel 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 Thyroxine 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-thyroxine-enzyme-immunoassay-kit-\(10\).assay](https://www.myassays.com/arbor-assays-thyroxine-enzyme-immunoassay-kit-(10).assay)

[https://www.myassays.com/arbor-assays-thyroxine-enzyme-immunoassay-kit-\(100\).assay](https://www.myassays.com/arbor-assays-thyroxine-enzyme-immunoassay-kit-(100).assay)



TYPICAL DATA

Sample	10 μ L Assay				100 μ L Assay			
	Mean OD	Net OD	% B/B0	Thyroxine Conc. (ng/mL)	Mean OD	Net OD	% B/B0	Thyroxine Conc. (pg/mL)
NSB	0.082	0.000	-	-	0.078	0.000	-	-
Standard 1	0.194	0.112	7.5	20	0.157	0.080	10.1	2,000
Standard 2	0.349	0.267	17.9	10	0.266	0.189	24.0	1,000
Standard 3	0.583	0.501	33.6	5	0.416	0.338	43.1	500
Standard 4	0.892	0.810	54.3	2.5	0.591	0.513	65.4	250
Standard 5	1.176	1.094	73.4	1.25	0.700	0.622	79.3	125
Standard 6	1.331	1.249	83.8	0.625	0.773	0.695	88.6	62.5
B0	1.573	1.491	100	0	0.862	0.785	100	0
Sample 1	0.90	0.819	54.9	2.48	0.335	0.257	32.8	717.9
Sample 2	1.233	1.151	77.2	1.00	0.556	0.479	61.0	284.8

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

Conversion Factor: 77.7 ng/mL of Thyroxine is equivalent to 100 nM.



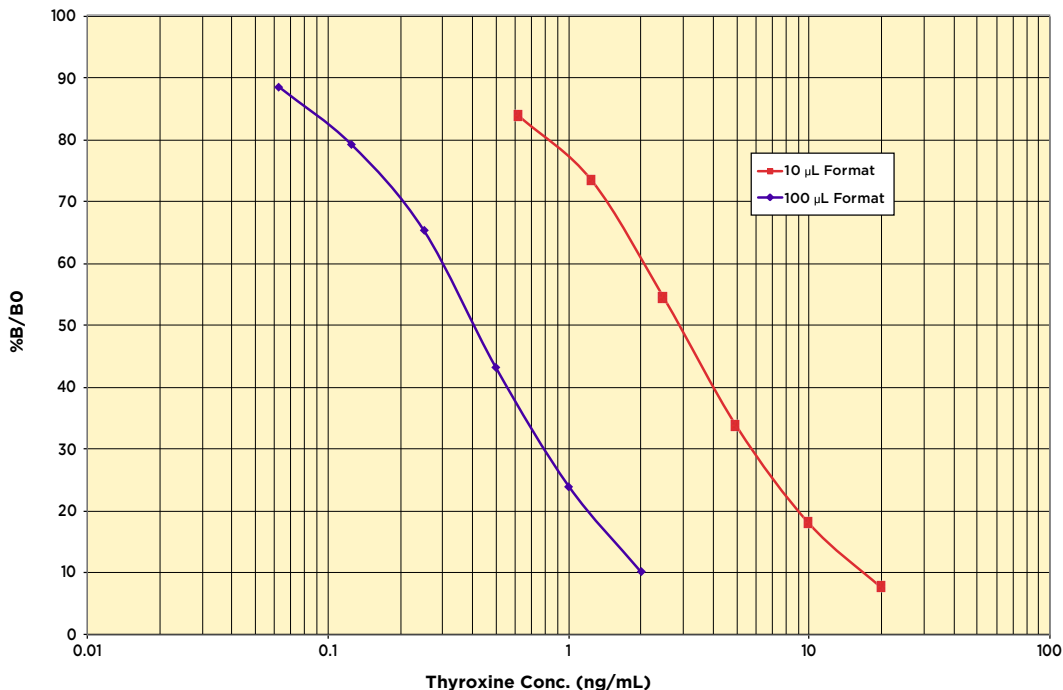
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Typical Standard Curves



**Always run your own standard curves for calculation of results.
Do not use these standard curves.**

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity with the 10 µL and the 100 µL sample volume 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 appropriate volume standard curve. **Sensitivity was determined as 0.23 ng/mL for 10 µL and 55.3 pg/mL for 100 µL sample size.**

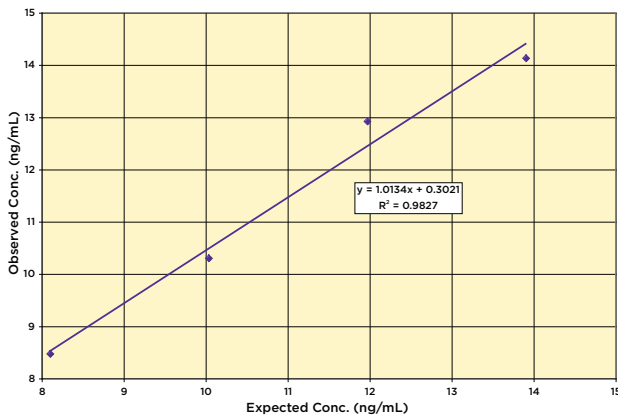
The Limit of Detection for the 10 µL and the 100 µL sample format was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample in the appropriate volume standard curve. **Limit of Detection was determined as 0.80 ng/mL for 10 µL and 47.4 pg/mL for 100 µL sample size.**

Linearity

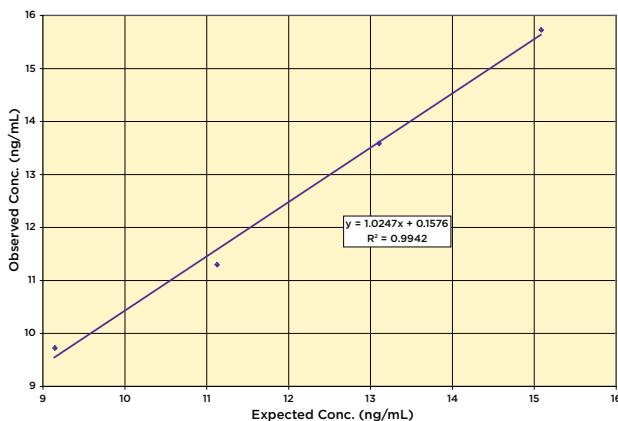
Linearity was determined for the 10 µL format using human serum and EDTA plasma samples, by taking samples with a high known thyroxine concentration and a lower thyroxine concentration and mixing them in the ratios given below. The measured thyroxine concentrations were compared to the expected values based on the ratios used.

High Sample	Low sample	Expected Conc. (ng/mL)		Observed Conc. (ng/mL)		% Recovery	
		Serum	Plasma	Serum	Plasma	Serum	Plasma
80%	20%	13.91	15.09	14.13	15.72	101.6	104.2
60%	40%	11.97	13.11	12.92	13.58	107.9	103.6
40%	60%	10.04	11.13	10.30	11.29	102.6	101.4
20%	80%	8.10	9.15	8.47	9.72	104.5	106.2
		Mean Recovery				104.2%	103.9%

Serum Linearity



Plasma Linearity



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Intra Assay Precision - 10 µL sample

Two human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated thyroxine concentrations were:

Sample	Thyroxine Conc. (ng/mL)	%CV
1	5.5	3.0
2	3.3	2.9

Inter Assay Precision - 10 µL sample

Two 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 thyroxine concentrations were:

Sample	Thyroxine Conc. (ng/mL)	%CV
1	5.4	6.3
2	3.3	7.8

SAMPLE VALUES

Twenty-two random mammalian serum samples were tested in the assay. Diluted sample values ranged from 1.4 to 6.2 ng/mL. Adjusted values for the samples ranged from 57.72 to 123.4 ng/mL with an average of 87.88 ng/mL. The normal reference range for serum thyroxine is 50-125 ng/mL¹.

Twelve random mammalian EDTA plasma samples were tested in the assay. Diluted sample values ranged from 3.66 to 7.96 ng/mL. Adjusted values for the samples ranged from 73.1 to 159.2 ng/mL with an average of 117.7 ng/mL.

Two random urine samples were tested in the assay. Adjusted values for the samples were 386.6 and 450 pg/mL.

Eight dried fecal samples were processed as described on page 7 and run in the assay. Iberian lynx and lion samples, kindly donated by Professor Martin Dehnhard of the Leibniz Institute for Zoo & Wildlife Research, Berlin and Dr. J. Williams of the Indianapolis Zoo, were tested and thyroxine values obtained ranged from 3.5 to 20.9 ng/mL.

1. Mayo Medical Labs.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Thyroxine	100%
Reverse T3 (3,3',5'-Triiodo-L-thyronine)	89.0%
T ₃ (3,3',5-Triiodo-L-thyronine)	5.23%



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.

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