ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

DNA DAMAGE
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K059-H1
5 Plate Kit Catalog Number K059-H5

Species Independent

Sample Types Validated:

**Serum, EDTA and Heparin Plasma,
Saliva, Urine, Digested DNA,
Fecal Extracts and Tissue Culture Media**

Patent Pending

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

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TABLE OF CONTENTS

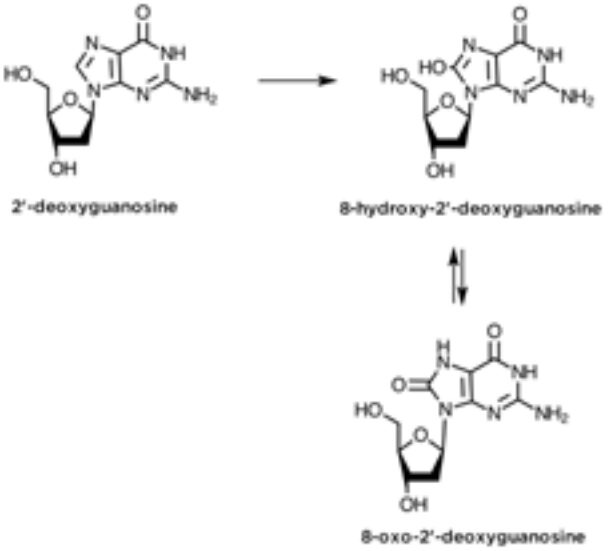
BACKGROUND

Free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. Intracellular free radical species (ROS) are produced as a result of normal metabolism and extracellular forms are produced as a result of ultraviolet radiation or ionizing radiation.

Cellular function may be interrupted or stopped if DNA damage corrupts the integrity of essential information contained in the genome. When individual bases are damaged, nonspecific DNA repair enzymes excise DNA lesions to release deoxynucleotides, and base specific repair glycosylases excise the corresponding base. Deoxynucleotides are enzymatically hydrolyzed to stable deoxynucleosides, and these repair products are transported through the blood and excreted in the urine. Damage to RNA is reflected in nucleoside adducts.

It is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of oxidative DNA damage, the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a ubiquitous marker of oxidative stress. 8-OHdG is physiologically formed and enhanced by chemical carcinogens. During the repair of damaged DNA *in vivo* by exonucleases, the resulting 8-OHdG is excreted without further metabolism into urine.

Reaction Scheme



ASSAY PRINCIPLE

The DetectX® DNA Damage Immunoassay Kit is designed to quantitatively measure DNA and RNA oxidized guanosine species. The assay detects all three oxidized guanine species; 8-hydroxy-2'-deoxyguanosine from DNA, 8-hydroxyguanosine from RNA and 8-hydroxyguanine, from digested DNA from DNA or RNA. These species may be present in serum, plasma, saliva, urine, dried fecal samples, and tissue culture media samples. Please read the complete kit insert before performing this assay.

An 8-hydroxy-2'-deoxyguanosine (8-OHdG) stock solution 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 8-hydroxyguanosine conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a peroxidase-labeled mouse monoclonal antibody to 8-hydroxy-2'-deoxyguanosine to each well. After an hour incubation the plate is washed and substrate added. The substrate reacts with the peroxidase labeled antibody that has reacted with the bound 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 450nm wavelength. The concentration of the 8-hydroxy-2'-deoxyguanosine 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.
Catalase Fluorescent Activity Kit	K033-F1
Catalase Colorimetric Activity Kit	K033-H1
Formaldehyde Fluorescent Detection Kit	K001-F1
FRAP™ Ferric Reducing Antioxidant Power Detection Kit	K043-H1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione Reductase Fluorescent Activity Kit	K009-F1
Hemoglobin Dual Range Detection Kit	K013-H1
Hydrogen Peroxide (H_2O_2) Fluorescent Detection Kit	K034-F1
Hydrogen Peroxide (H_2O_2) Colorimetric Detection Kit	K034-H1
Nitric Oxide (NO) Colorimetric Detection Kit	K023-H1
Superoxide Dismutase (SOD) Activity Kit	K028-H1

SUPPLIED COMPONENTS

Coated Clear 96 Well Plate
A clear plastic microtiter plate(s) with 1x8 strips coated with goat anti-rabbit IgG.

Kit K059-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

8-Hydroxy-2'-deoxyguanosine Standard
8-hydroxy-2'-deoxyguanosine at 160 ng/mL in a special stabilizing solution.

Kit K059-H1 **or** -H5	70 µL **or** 350 µL	Catalog Number C222-70UL **or** -350UL

DetectX® 8-Hydroxy-2'-deoxyguanosine Antibody
A mouse monclonal antibody labeled with peroxidase in a special stabilizing solution.

Kit K059-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C220-3ML **or** -13ML

DetectX® 8-Hydroxyguanosine EIA Conjugate
A 8-hydroxyguanosine conjugate in Assay Buffer.

Kit K059-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C221-3ML **or** -13ML

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

Kit K059-H1 **or** -H5	28 mL **or** 55 mL	Catalog Number X131-28ML **or** -55ML

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

Kit K059-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K059-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K059-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K059-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 µL, 50 µL and 100 µL.

Plate 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.

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 serum, EDTA or heparin plasma, saliva, and urine samples, and for digested DNA and 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.

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is identical across all species and we expect this kit may measure 8-OHdG in all other species. The end user should evaluate recoveries of 8-OHdG in the samples being tested.

SAMPLE PREPARATION

8-Hydroxy-2'-deoxyguanosine can be assayed in other sample types by using one of the extraction protocols available on our website at: www.arborassays.com/resources/#protocols

Serum and Plasma Samples

Serum and plasma samples should be diluted ≥ 1:8 with the diluted Assay Buffer prior to running in the assay.

Saliva Samples
Saliva samples should be diluted ≥ 1:2 with the diluted Assay Buffer prior to running in the assay.

Urine Samples
Urine samples should be diluted ≥ 1:4 with the diluted Assay Buffer prior to running in the assay. Please see our Urinary Creatinine Detection kits, K002-H1 and K002-H5, for assays to measure urine creatinine which can be used to normalize 8-hydroxy-2'-deoxyguanosine concentrations in a random urine specimen.

Dried Fecal Samples
Use the detailed Steroid Solid Extraction Protocol that is available on our website at: www.arborassays.com/resources/#protocols for determining 8-hydroxy-2'-deoxyguanosine in fecal samples. The ethanol concentration in the final Assay Buffer dilution added to the well should be ≤ 2.5%.

DNA Samples
Purify DNA from cell or tissue samples by a desired method or commercial DNA purification kit, such as QiaAMP DNA Mini Kit (Qiagen 51304 or 51306).

Reagents needed but not supplied: Nuclease P1 (Sigma-Aldrich N8630), 3M Sodium Acetate, pH 5.2, 1M Tris pH 7.5, Alkaline Phosphatase (NEB M02905), Zinc Chloride (Sigma-Aldrich 39059).

For digestion, 15µg of DNA in 100µL DNA hydration buffer or DI water is required. Protocol volumes can be scaled.

1. Prepare working solution of Nuclease P1 at 5U/mL in 40mM sodium acetate. Keep on ice. Remove aliquot of alkaline phosphatase, 10U/mL, from -20˚C. Keep on ice. Thaw normalized DNA samples (15µg/100µL).

2. Denature the DNA at 95-100˚C for 10 min. Cool completely on ice 5 min. Centrifuge for 5 sec or tap any condensate down into tube. Add 50µL 40mM sodium acetate pH 5.0-5.4, 0.4mM $ZnCl_2$.

3. Add 50µL of 5U/mL Nuclease P1. Invert tube to mix. Centrifuge 5 seconds or tap any condensate down into tube. Incubate at 37˚C for 30 min.

4. Adjust pH to 7.5-8.0 by adding 20µL 1M Tris pH 7.5 to tube. Add 15µL of 10U/mL alkaline phosphatase. Invert to mix. Centrifuge 5 sec or tap any condensate down into tube.

5. Incubate at 37˚C for 30 min. Boil samples for 10 min at 95˚C to inactivate alkaline phosphatase. Place samples on ice. Aliquot samples, 2µg/tube and store at ≤ -20˚C until assaying. Samples should be diluted ≥ 1:4 with the diluted Assay Buffer prior to running in the assay.

Tissue Culture Media
For measuring 8-hydroxy-2'-deoxyguanosine in tissue culture media (TCM), samples should be prepared with the diluted Assay Buffer prior to running in the assay. We have validated the assay using RPMI-1640. The end user should determine the appropriate dilution for their TCM.

Use all Samples within 2 hours of preparation, or stored at ≤ -20°C until assaying.

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

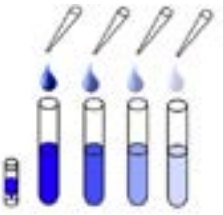
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable for 3 months at 4°C.

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 for 3 months at room temperature.

Standard Preparation

Label test tubes as #1 through #8. Pipet 475 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #8. Carefully add 25 µL of the 8-hydroxy-2'-deoxyguanosine stock solution to tube #1 and vortex completely. Take 250 µL of the 8-hydroxy-2'-deoxyguanosine solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of 8-hydroxy-2'-deoxyguanosine in tubes 1 through 8 will be 8,000, 4,000, 2,000, 1,000, 500, 250, 125, and 62.5 pg/mL.



Use all Standards within 2 hour of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**475**	250	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**25**	250	250	250	250	250	250	250
Final Conc (pg/mL)	8,000	4,000	2,000	1,000	500	250	125	62.6

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine 8-Hydroxy-2'-deoxyguanosine 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® 8-Hydroxy-2'-deoxyguanosine Conjugate to each well, **except the NSB wells**, using a repeater pipet.
6. Add 25 µL of the DetectX® 8-Hydroxy-2'-deoxyguanosine Antibody to each well 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 15-20% 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 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 8-hydroxy-2'-deoxyguanosine 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-dna-damage-eia-kit.assay

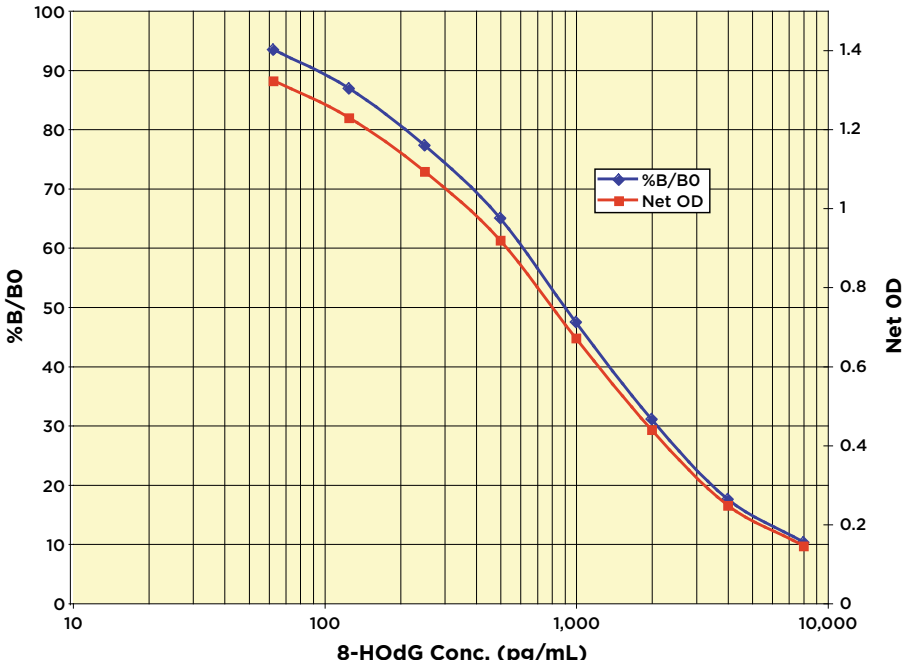
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	8-Hydroxy-2'-deoxyguanosine Conc. (pg/mL)
NSB	0.119	0.000		-
Standard 1	0.264	0.145	10.2	8,000
Standard 2	0.367	0.248	17.5	4,000
Standard 3	0.558	0.439	31.0	2,000
Standard 4	0.790	0.671	47.4	1,000
Standard 5	1.038	0.919	64.9	500
Standard 6	1.212	1.093	77.2	250
Standard 7	1.348	1.229	86.9	125
Standard 8	1.441	1.322	93.4	62.5
B0	1.534	1.415	100	0
Sample 1	1.136	1.017	71.9	348.8
Sample 2	0.786	0.667	47.1	1,012

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

Conversion Factor: 100 pg/mL of 8-hydroxy-2'-deoxyguanosine is equivalent to 353.0 pM.

Typical Normal Range 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 eighteen wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 50.9 pg/mL.

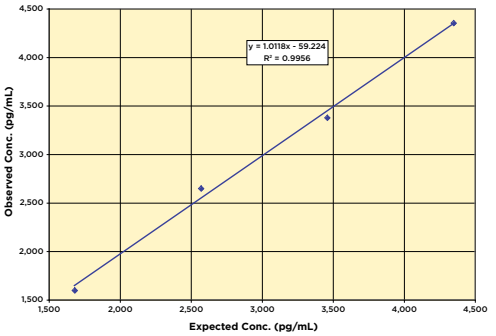
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for eighteen runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 82.2 pg/mL.

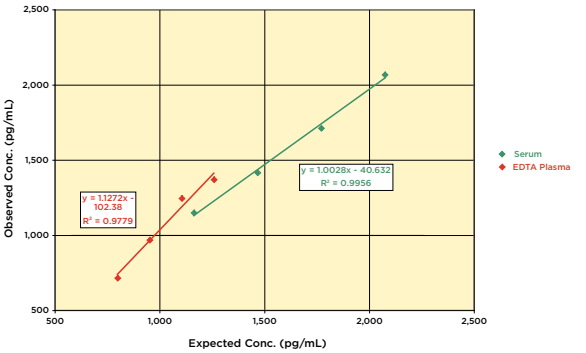
Linearity

Linearity in urine was determined by taking two samples, one with a low diluted 8-hydroxy-2'-deoxyguanosine level of 793.4 pg/mL and one with a higher diluted level of 5,238 pg/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 serum and EDTA plasma were determined in a similar manner.

	High Sample	Low Sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
Urine	80%	20%	4,350	4,351	100.0
	60%	40%	3,460	3,375	97.5
	40%	60%	2,571	2,647	102.9
	20%	80%	1,682	1,596	94.8
				Mean Urine Recovery	**98.8%**
Serum	80%	20%	1,311	1,338	102.0
	60%	40%	1,128	1,223	108.4
	40%	60%	945.8	968.4	102.4
	20%	80%	763.2	736.7	96.5
				Mean Serum Recovery	**102.3%**
EDTA Plasma	80%	20%	2,076	2,065	99.5
	60%	40%	1,772	1,709	96.4
	40%	60%	1,468	1,414	96.3
	20%	80%	1,164	1,148	98.6
				Mean Plasma Recovery	**97.7%**



Urine Linearity



Serum and Plasma Linearity

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 8-hydroxy-2'-deoxyguanosine concentrations were:

Sample	8-Hydroxy-2'-deoxyguanosine Conc. (pg/mL)	%CV
1	423.6	11.7
2	995.7	8.2
3	1,187	7.1

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in nineteen assays run over multiple days by five operators. The mean and precision of the calculated 8-hydroxy-2'-deoxyguanosine concentrations were:

Sample	8-Hydroxy-2'-deoxyguanosine Conc. (pg/mL)	%CV
1	404.9	13.4
2	887.8	8.3
3	1,121	8.1

SAMPLE VALUES

Ten human serum samples were tested in the assay. Neat sample values ranged from approximately 9,300 to over 20,300 pg/mL with an average of 14,584 pg/mL.

Ten human EDTA plasma samples were tested in the assay. Neat sample values ranged from approximately 5,735 to almost 12,000 pg/mL with an average of 8,183 pg/mL.

Two human saliva samples were tested in the assay. Neat sample values were 3,580 and 7,190 pg/mL with an average of 5,386 pg/mL.

Thirteen human urine samples were tested in the assay. Neat sample values ranged from 26.0 to over 212 ng/mL with an average of 92.1 ng/mL.

Two digested DNA samples were tested in the assay. Neat sample values were 46.6 and 78.5 pg 8-HOdG/µg DNA with an average of 62.5 pg 8-HOdG/µg DNA.

Four mammalian fecal extracts were tested in the assay. Neat sample values ranged from 121.6 to 527.1 pg/mg dry fecal weight with an average of 315.9 pg/mg dry fecal weight

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
8-Hydroxy-2'-deoxyguanosine	100%
8-Hydroxyguanosine	27.32
8-Hydroxyguanine	9.50

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

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

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