ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
MOST SENSITIVE
N-CAL™ KIT

NCal™ International Standard Kit

DetectX®

Arg[8]-Vasopressin

Chemiluminescent Immunoassay Kit

1 Plate Kit Catalog Number K049-C1
5 Plate Kit Catalog Number K049-C5

Species Independent

Sample Types Validated:

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.

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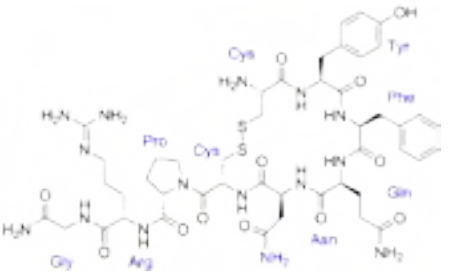
BACKGROUND

The neuropeptides, oxytocin and vasopressin, were isolated and synthesized by Vincent du Vigneaud at Cornel Medical College in 1953, work for which he received the Nobel Prize in Chemistry in 1955[1]. The neurohypophysial hormone arginine vasopressin (AVP), which is also known as an antidiuretic hormone, is involved in a wide range of physiological regulatory processes, including renal water reabsorption, cardiovascular homeostasis, hormone secretion from the anterior pituitary, and modulation of social behavior and emotional status[2]. AVP and the structurally related posterior pituitary hormone, oxytocin (OT), are synthesized in the paraventricular nucleus and the supraoptic nucleus of the hypothalamus[3]. AVP is a 9 amino acid peptide with a 6-member disulfide ring. It is structurally related to oxytocin differing by 2 amino acids.

AVP is released in response to sexual stimulation, uterine dilatation, stress, and dehydration. AVP V_2 receptors



Arg[8]-Vasopressin

in the kidney are antidiuretic, whereas the receptors V_{1a} and V_{1b} receptors in the vascular tree, adrenal gland, uterus, and other tissues mediate the diverse peripheral effects of this peptide[4]. AVP acts principally on renal collecting tubules to increase water reabsorption. Diabetes insipidus (DI) is characterized by the inability to appropriately concentrate urine in response to volume and osmol stimuli. The main causes for DI are decreased AVP production (central DI) or decreased renal response to AVP (nephrogenic DI). AVP can also be secreted inappropriately in certain situations, particularly in elderly patients, leading to water retention and dilutional hyponatremia. Inappropriate AVP secretion might be observed with central nervous system pathology, such as head injury, stroke, or cerebral tumor, or as a side effect of central acting drugs that interfere with the hypothalamic regulation or AVP. Noncentral causes of inappropriate AVP secretion include peripheral stimuli that mimic central vascular hypovolemia, in particular severe low-output cardiac failure, and ectopic AVP secretion (usually by a bronchogenic carcinoma).

1. Ragnarsson U., “The nobel trail of Vincent du Vigneaud”., J. Pept. Sci., 2007, 13:431–433.
2. Laycock JF., Perspectives on Vasopressin., Singapore: World Scientific, 2010.
3. Armstrong WE., “Hypothalamic supraoptic and paraventricular nuclei.”, In: The Rat Nervous System, edited by Paxinos G. San Diego, CA: Elsevier Academic, 2004, p.369–388.
4. Insel, TR., “The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior”, 1995, NY: Plenum Press.

ASSAY PRINCIPLE

The DetectX® Arg[8]-Vasopressin (AVP) Chemiluminescent Immunoassay Kit is designed to quantitatively measure AVP present in serum, plasma and tissue culture media samples. Please read the complete kit insert before performing this assay.

An AVP 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 white microtiter plate coated with an antibody to capture rabbit antibodies. An AVP-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 AVP to each well. After an overnight incubation at 4°C the plate is washed and supplied substrate is added. The substrate reacts with the bound AVP-peroxidase conjugate. The intensity of the generated chemiluminescent signal is detected in a microtiter plate reader capable of measuring luminescence. The concentration of the AVP 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.
Acetylcholinesterase Fluorescent Activity Kit	K015-F1
Butyrylcholinesterase Fluorescent Activity Kit	K016-F1
Corticosterone ELISA Kits	K014-H1/H5
Corticosterone Chemiluminescent ELISA Kits	K014-C1/C5
Serum Creatinine Detection Kits	KB02-H1/H2/H1D
Cystatin C Human ELISA Kit	K012-H1
Progesterone ELISA Kits	K025-H1/H5
Progesterone Metabolites ELISA Kits	K068-H1/H5
Oxytocin ELISA Kits	K048-H1/H5
Oxytocin Chemiluminescent ELISA Kits	K048-C1/C5
Retinol Binding Protein (RBP) Multi-Format ELISA Kits	K062-H1/H5

SUPPLIED COMPONENTS

Coated White 96 Well Plates
White plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG.
Kit K049-C1 **or** -C5 1 **or** 5 Each — Catalog Number X014-1EA

Arg-Vasopressin Standard
AVP at 100,000 pg/mL in a special stabilizing solution.
Calibrated to the U.S. Pharmacopeial Convention Cat. No.: 1711100 Lot: H0L444
Kit K049-C1 **or** -C5 25 µL **or** 125 µL — Catalog Number C183-25UL **or** -125UL

DetectX® Arg-Vasopressin Antibody
A rabbit polyclonal antibody specific for AVP in a special stabilizing solution.
Kit K049-C1 **or** -C5 3 mL **or** 13 mL — Catalog Number C172-3ML **or** -13ML

DetectX® Arg-Vasopressin Conjugate
AVP-peroxidase conjugate in a special stabilizing solution.
Kit K049-C1 **or** -C5 3 mL **or** 13 mL — Catalog Number C173-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
Kit K049-C1 **or** -C5 28 mL **or** 55 mL — Catalog Number X065-28ML **or** -55ML

Extraction Solution
A special extraction solution for treatment of serum and plasma samples to extract AVP.
Kit K049-C1 **or** -C5 50 mL **or** 250 mL — Catalog Number X123-50ML **or** -250ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
Kit K049-C1 **or** -C5 30 mL **or** 125 mL — Catalog Number X007-30ML **or** -125ML

Substrate Solution A
Kit K049-C1 **or** -C5 6 mL **or** 28 mL — Catalog Number X077-6ML **or** -28ML

Substrate Solution B
Kit K049-C1 **or** -C5 6 mL **or** 28 mL — Catalog Number X078-6ML **or** -28ML

Plate Sealer
Kit K049-C1 **or** -C5 1 **or** 5 Each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

This kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

A Speedvac or other centrifugal vacuum concentrator or a manifold and inert gas supply, such as nitrogen or helium, to evaporate extracted samples.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25 µL, 50 µL, and 100 µL.

A microplate shaker.

96 well microplate reader capable of reading glow chemiluminescence. A list of some models of suitable readers can be found on our website at www.arborassays.com/resources/#general-info. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. **The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol:**

> Dilute 5 µL of the AVP Conjugate Concentrate into 495 µL of deionized water. Pipet 5 µL of this dilution into an uncoated white well and add 100 µL of prepared CLIA substrate (see page 8 for details). This well will give you an intensity 0.7-0.8 times the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the readers maximum signal.

To properly analyze the data, software will be required for converting raw RLU 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.

SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma, and tissue culture samples. Samples containing visible particulate matter should be centrifuged before use. Platelet poor EDTA plasma is recommended for AVP measurements and should be collected according to the protocol from WHO International Agency for Research on Cancer working group report, page 24 at: www.iarc.fr/en/publications/pdfs-online/wrk/wrk2/standardsBRC-8.pdf

AVP is identical across almost all species and we expect this kit may measure AVP from a wide variety of sources other than human. Pigs have Lys^{8}-vasopressin (LVP) instead of AVP and as this assay has essentially zero reactivity to LVP porcine samples should not be used. This assay has 26.2% cross reactivity for Arg^{8}-vasotocin, the reptile and avian analogue of Arg^{8}-vasopressin. It will therefore be useful in measuring samples from most species including mammals, reptiles, fish and birds. The end user should evaluate recoveries of AVP in other samples being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples should be extracted with the provided Extraction Solution, or with a solid phase C18 column extraction protocol (see Peptide/Protein Extraction Protocol at www.arborassays.com/resources/#protocols) prior to running in the kit.

Protocol Using Extraction Solution:

1. Mix 1 part sample with 1.5 parts of Extraction Solution.
2. Vortex and then nutate at room temperature for 90 minutes.
3. Centrifuge for 20 minutes at 4°C at 1660 x g.
4. Transfer supernatant to a clean tube.
5. Speedvac supernatant to dryness at 37°C.
6. Reconstitute sample with 250 µL of Assay Buffer.

Use all samples within 2 hour 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

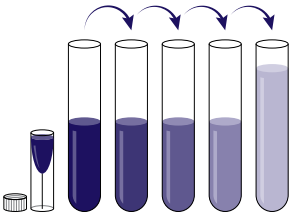
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 #8. Pipet 990 µL of Assay Buffer into tube #1 and 300 µL into the remaining tubes. **The Arg-Vasopressin stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 µL of the AVP stock solution to tube #1 and vortex completely. Take 200 µL of the AVP 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 AVP will be 1,000, 400, 160, 64, 25.6, 10.24, 4.096 and 1.638 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**990**	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	200	200	200	200	200	200	200
Final Conc (pg/mL)	1,000	400	160	64	25.6	10.24	4.096	1.638

Chemiluminescent Substrate

Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle. Once mixed the substrate is stable for one month when stored at 4°C.

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine AVP 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 100 µL of samples or standards into wells in the plate.
3. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
4. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
5. Add 25 µL of the DetectX® AVP Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® AVP Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. We recommend shaking at around 700–900 rpm. Cover the plate with the plate sealer and store at 4°C for 16-18 hours.
8. The following day remove the Chemiluminescent Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**
9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 5 minutes without shaking.
12. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.
13. Use the plate reader's built-in 4PLC software capabilities to calculate AVP 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

All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU 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 RLU'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-arg8-vasopressin-clia-kit.assay

TYPICAL DATA

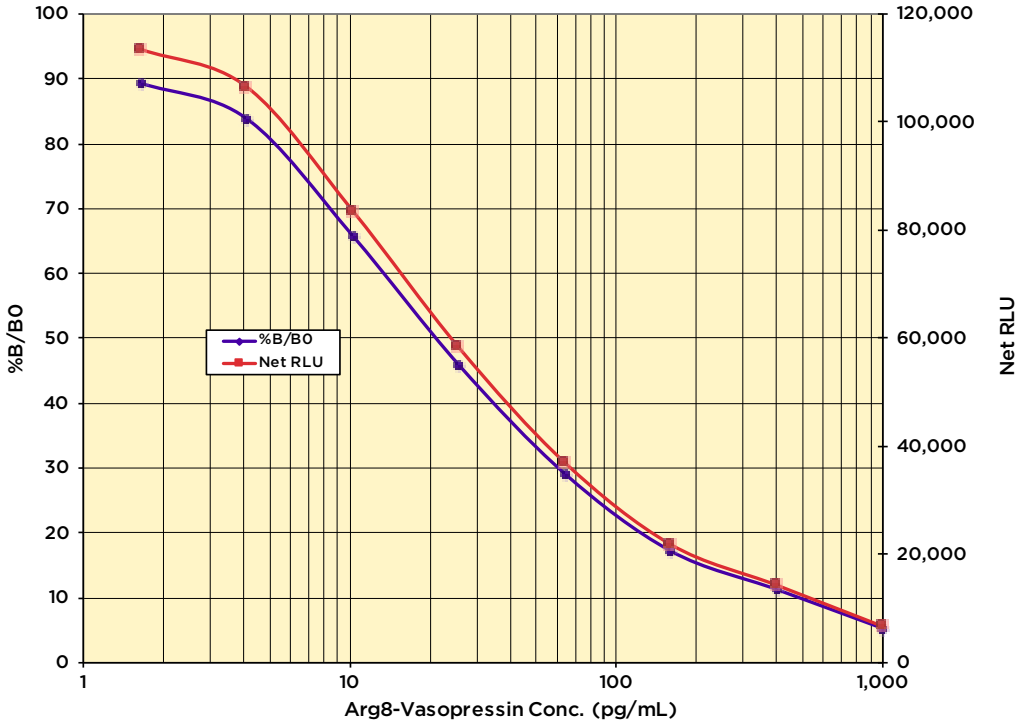
Sample	Mean RLU	NetRLU	% B/B0	AVP Conc. (pg/mL)
NSB	14,140	0	-	-
Standard 1	20,865	6,725	5.30	1,000
Standard 2	28.485	14,345	11.29	400
Standard 3	36,020	21,880	17.23	160
Standard 4	51,180	37,040	29.16	64
Standard 5	72,495	58,355	45.95	25.6
Standard 6	97,775	83,635	65.85	10.24
Standard 7	120,690	106,550	83.89	4.096
Standard 8	127,635	113,495	89.36	1.638
B0	141,145	127,005	100.0	0
Sample 1	62,125	47,985	37.78	39.7
Sample 2	95,780	81,640	64.28	11.4

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

Conversion Factor: 1 ng/mL of AVP is equivalent to 0.922 nM.

Calibrated to the U.S. Pharmacopeial Convention Cat. No.: 1711100 Lot: H0L444

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 RLUs for twenty wells run for each of the RLUs and standard #8. The detection limit was determined at two (2) standard deviations from the RLUs along the standard curve.

Sensitivity was determined as 0.88 pg/mL.

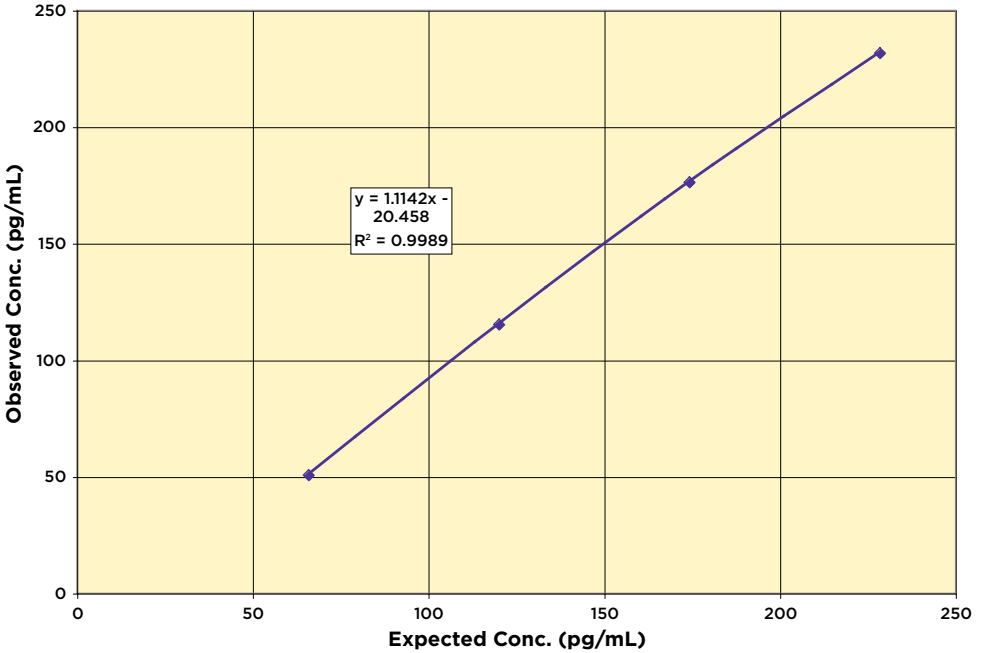
The Limit of Detection for the assay was determined in a similar manner by comparing the RLUs for twenty runs for each of the zero standard and a low concentration sample.

Limit of Detection was determined as 1.16 pg/mL.

Linearity

Linearity was determined by taking two samples, one with a low level of 11.6 pg/mL and one with a higher level of AVP of 282.7 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 Sample	Low Sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	228.5	231.8	101.4%
60%	40%	174.3	176.3	101.1%
40%	60%	120.1	115.3	96.0%
20%	80%	65.9	50.8	77.1%
			Mean Recovery	**93.9%**



Intra Assay Precision

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

Sample	AVP Conc. (pg/mL)	%CV
1	293.1	11.1%
2	43.0	7.4%
3	10.4	9.9%

Inter Assay Precision

Two samples were diluted with Assay Buffer and run in duplicates in 19 assays run over multiple days by four operators. The mean and precision of the calculated AVP concentrations were:

Sample	AVP Conc. (pg/mL)	%CV
1	271.1	16.9%
2	39.8	10.4%
3	10.3	16.1%

SAMPLE VALUES

Human AVP concentrations in platelet poor EDTA plasma are typically less than 1.7 pg/mL[5]. A number of conditions will cause an elevation in AVP levels, including type 1 diabetes, seizures, cerebral hemorrhages, cerebral trauma, cerebral tumors, neurosurgery, electroconvulsive therapy, central nervous system acting drugs, and a variety of conditions that reduce apparent blood volume or pressure in central vessels can all result in inappropriate AVP elevations[5].

A significant amount of circulating AVP is associated with platelets. Therefore, various conditions affecting platelets may also affect AVP levels. Platelet-rich specimens have been shown to have AVP levels on the order of 10 times the value of platelet-poor specimens.

We ran numerous human serum and EDTA plasma samples, extracted according to the protocol outlined on page 7. Serum values from healthy individuals ranged from 10.44 to 12.54 with an average of 11.04 pg/mL. Human EDTA plasma AVP concentrations were measured from 4.63 to 9.64 with a mean of 7.04. No platelet poor EDTA plasma samples were run.

5. Mayo Medical Laboratories, Arginine Vasopressin, Plasma reference. www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/80344

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Arg^{8}-Vasopressin	100%
Arg^{8}-Vasotocin	26.2%
Desmopressin	0.56%
Oxytocin	0.06%
Isotocin	< 0.01%
Lys^{8}-Vasopressin	< 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.

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FOR SERIOUS SCIENCE

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