DetectX®

Serum Retinol Binding Protein
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K004-H1

Species Independent

Sample Types Validated:

Serum, EDTA and Heparin Plasma, Dried Blood Spots

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	3
Assay Principle	4
Related Products	4
Supplied Components	5
Storage Instructions	5
Other Materials Required	6
Precautions	6
Sample Types	7
Sample Preparation	7
Reagent Preparation	8
Assay Protocol	9
Calculation of Results	10
Typical Data	10-11
Validation Data Sensitivity, Linearity, etc.	11-13
Samples Values and Cross Reactivity	14
Warranty & Contact Information	15
Plate Layout Sheet	16

BACKGROUND

Retinol binding protein (RBP) is from a family of structurally related proteins that bind small hydrophobic molecules such as bile pigments, steroids, odorants, etc[1]. RBP is a 21 kDa highly conserved, single-chain glycoprotein, consisting of 182 amino acids with 3 disulfide bonds, that has a hydrophobic pocket which binds retinol (vitamin A). The structure of retinol is shown below.

RBP binds retinol in a 1:1 stoichiometry, which serves to not only solubilize retinol but also protect it from oxidation. When in serum, the majority of RBP bound with retinol is reversibly complexed with transthyretin (prealbumin)[2]. This complex then transports retinol to specific receptors of various tissues in the body. Vitamin A status is reflected by serum concentration as it is hemostatically controlled and does not fall until stores are dramatically reduced[3-4].

RBP has been shown to be a useful surrogate marker for retinol because of the approximate 1:1 (molar) correlation between retinol and RBP in serum[1, 5-6], which implies that RBP may be used to assess and monitor vitamin A deficiency (VAD) in populations. The World Health Organization has estimated that 250 million children have moderate to severe VAD[7] due to lack of adequate nutrition, and the rising cost of food staples around the world further exacerbates this problem. In addition to nutritional deficiencies, infectious stresses have been shown to depress retinol concentrations. Therefore, individuals with diseases such as cystic fibrosis[8] and HIV-1[9] also run the risk of VAD due to the infectious stresses that contribute to the disease.

1. Blaner WS. “Retinol binding protein: the serum transport protein for vitamin A.” Endocr Rev. 1989 Aug;10(3):308–16.
2. Wolf G. “Multiple functions of vitamin A.” Physiol. Rev. 1984 Jul;64(3):873-937.
3. Goodman DS, Blaner WS. in The Retinoids, eds. Sporn MB, Roberts AB, Goodman DS, (Orlando: Academic Press, 1984) vol.2, 1-39.
4. Olson, JA. “Vitamin A, retinoids and carotenoids.” Modern Nutrition in Health and Disease, eds. Shils ME, Olson JA, Shike M, (Philadelphia: Lea & Febiger, 1994) 8th ed. 287–307.
5. Almekinder J, Manda W, Soko D, Lan Y, Hoover DR, Semba RD. “Evaluation of plasma retinol-binding protein as a surrogate measure for plasma retinol concentrations.” Scand J Clin Lab Invest. 2000 May;60(3):199-203.
6. Gamble MV, Ramakrishnan R, Palafox NA, Briand K, Berglund L, Blaner WS. “Retinol binding protein as a surrogate measure for serum retinol: studies in vitamin A–deficient children from the Republic of the Marshall Islands.” Am J Clin Nutr. 2001 May;73(3):594–601.
7. World Health Organization, Division of Nutrition, “Global Prevalence of Vitamin A Deficiency,” Micro nutrient Deficiency Information System, No.2 (1995) Geneva: WHO/UNICEF.
8. Duggan C, Colin AA, Agil A, Higgins L, Rifai N. “Vitamin A status in acute exacerbations of cystic fibrosis.” Am J Clin Nutr. 1996 Oct;64(4):635-9.
9. Baeten JM, Richardson BA, Bankson DD, Wener MH, Kreiss JK, Lavreys L, Mandaliya K, Bwayo JJ, McClelland RS. “Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response.” Am J Clin Nutr. 2004;79:218-25.

ASSAY PRINCIPLE

The DetectX® Retinol Binding Protein (RBP) Immunoassay Kit is designed to quantitatively measure RBP present in serum, plasma or dried blood spot samples. Please read the complete kit insert before performing this assay. This kit measures all isoforms of RBP.

The kit offers 2 standard curve ranges.

- For serum and plasma samples with normal RBP levels we recommend using 10 µL of standards or samples. The assay concentration range for RBP will be from 5,000 ng/mL to 39.1 ng/mL.
- For samples where RBP may be very low we recommend alternatively using 25 µL of standards or samples for an assay range of 2,500 ng/mL to 19.5 ng/mL

Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. RBP-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of the RBP polyclonal antibody to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound RBP-peroxidase conjugate. After a 30 minute 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 RBP in the sample is calculated, after making a suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCT

Kits	Catalog No.
Hemoglobin Colorimetric Detection Kit (2 Plate)	K013-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5

Watch our EIA video at:
www.ArborAssays.com/resources/#videos

SUPPLIED COMPONENTS

Coated Clear 96 Well Plate
A clear plastic microplate with break-apart strips coated with goat anti-rabbit IgG.
1 Each — Catalog Number X016-1EA

RBP Standard
A stock solution of native human RBP at 100 µg/mL.
60 µL — Catalog Number C009-60UL

DetectX® RBP Antibody
A polyclonal antibody specific for RBP.
3 mL — Catalog Number C011-3ML

DetectX® RBP-Peroxidase Conjugate
A RBP-peroxidase conjugate in a special stabilizing solution.
5 mL — Catalog Number C010-5ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
28 mL — Catalog Number X053-28ML

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

TMB Substrate
11 mL — Catalog Number X019-11ML

Stop Solution
A 1M hydrochloric acid solution. **Caustic**.
5 mL — Catalog Number X020-5ML

Plate Sealer
1 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.

A microplate shaker and washer.

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.

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.

The RBP Standard is purified from a human source and as such, should be treated as potentially hazardous. Proper safety procedures must be followed.

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.

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 human serum, EDTA and heparin plasma and dried blood spot samples (DBS) only. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit. They have been shown to yield artificially high RBP concentrations.

For detecting RBP in urine samples please refer to the DetectX® Urinary Retinol Binding Protein Immunoassay kit, Catalog Number KU04-H1, that is 10 times more sensitive than this serum kit.

RBP is a highly conserved protein and we have shown that the RBP kits, which uses identical antibody and conjugate, will measure RBP's from sources other than human including rat, dog and rhesus monkey. The end user should evaluate recoveries of RBP in other plasma and serum samples being tested.

SAMPLE PREPARATION

Serum and Plasma

Serum and plasma samples must be diluted ≥ 1:40 by taking one part of serum or plasma and adding thirty-nine parts of diluted Assay Buffer prior to running in the kit.

Dried Blood Spots (DBS)

Dried blood spot (DBS) samples should be prepared according to the 2007 Clinical Chemistry paper by Masako Fujita, et al, vol. 53 (11), page 1972-1975. Briefly, whole blood is spotted onto Whatman 309 filter paper and thoroughly dried at room temperature. These can be stored desiccated at ≤ 4°C until use. DBS samples, 1/4" or 1/8", are punched out into clean plastic tubes with caps. For each 1/4" DBS a minimum of 240 µL of diluted Assay Buffer is added. For each 1/8" DBS a minimum of 60 µL of diluted Assay Buffer is added. This is equivalent to a 1:40 dilution of the sample. The tubes are capped and left at 4°C overnight. The following morning, the red solution can be run without centrifugation or further dilution.

The dilution of any samples that fall outside the standard range should be adjusted to allow samples to read within the standard curve.

Use all samples within 2 hours of dilution.

REAGENT PREPARATION

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine RBP concentrations. 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 Normal RBP VAD Samples

Label test tubes as #1 through #8. Pipet 190 µL of Assay Buffer into tube #1 and 100 µL into tubes #2 to #8. Carefully add 10 µL of the RBP stock solution to tube #1 and vortex completely. Take 100 µL of the RBP 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 RBP in tubes 1 through 8 will be 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125 and 39.06 ng/mL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**190**	100	100	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	100	100	100	100	100	100	100
Final Conc (ng/mL)	5,000	2,500	1,250	625	312.5	156.25	78.125	39.06

Standard Preparation - 25 µL Assay Format   For Low RBP VAD Samples

Label test tubes as #1 through #8. Pipet 390 µL of Assay Buffer into tube #1 and 100 µL into tubes #2 to #8. Carefully add 10 µL of the RBP stock solution to tube #1 and vortex completely. Take 100 µL of the RBP 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 RBP in tubes 1 through 8 will be 2,500, 1,250, 625, 312.5, 156.25, 78.125, 39.06 and 19.53 ng/mL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**390**	100	100	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	100	100	100	100	100	100	100
Final Conc (ng/mL)	2,500	1,250	625	312.5	156.25	78.125	39.06	19.53

Use all Standards within 2 hour of preparation.

ASSAY PROTOCOL - 10 µL AND 25 µL

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 µL (25 µL for alternate format) of samples or standards into wells in the plate.

3. Pipet 35 µL (50 µL for alternate format) of Assay Buffer into the non-specific binding (NSB) wells.

4. Pipet 10 µL (25 µL for alternate format) of Assay Buffer into the maximum binding (B0 or Zero standard) wells.

5. Add 50 µL of the DetectX® RBP Conjugate to each well using a repeater pipet.

6. Add 25 µL of the DetectX® RBP 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 µL of 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 RBP 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 computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean OD's for the blank. The sample concentrations, calculated off 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-retinol-binding-protein-serum-eia-kit.assay

TYPICAL DATA

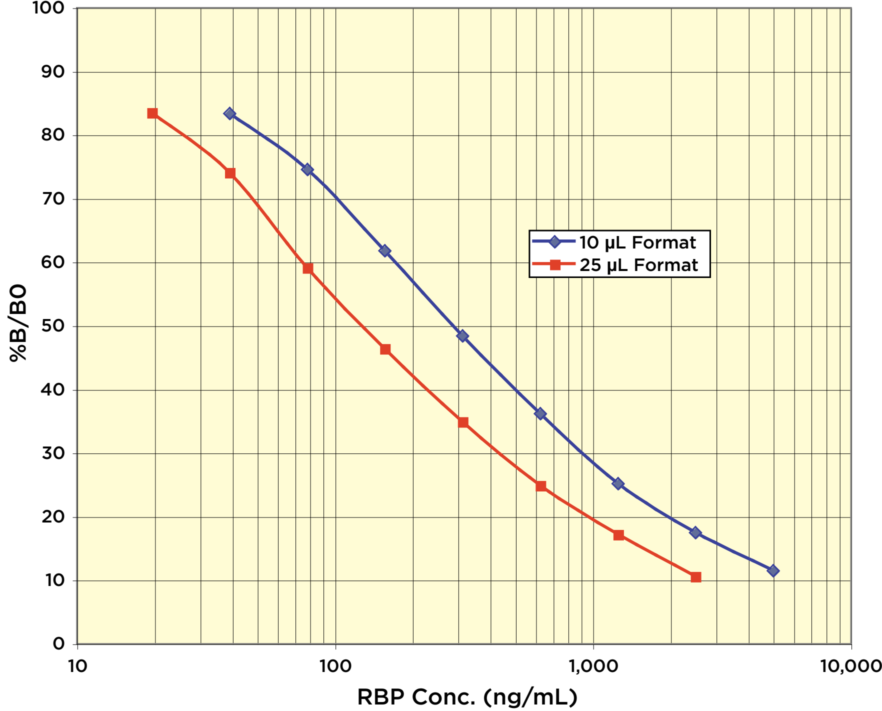
	10 µL Assay				25 µL Assay			
Sample	**Mean OD**	**Net OD**	**% B/B0**	**RBP Conc. (ng/mL)**	**Mean OD**	**Net OD**	**% B/B0**	**RBP Conc. (ng/mL)**
NSB	0.110	0.000	-	-	0.106	0.000	-	-
Standard 1	0.361	0.251	11.5	5,000	0.306	0.200	10.6	2,500
Standard 2	0.492	0.382	17.5	2,500	0.430	0.324	17.2	1,250
Standard 3	0.662	0.552	25.2	1,250	0.574	0.468	24.9	625
Standard 4	0.903	0.793	36.2	625	0.763	0.657	34.9	312.5
Standard 5	1.169	1.059	48.4	312.5	0.978	0.872	46.4	156.3
Standard 6	1.463	1.353	61.8	156.3	1.217	1.111	59.1	78.125
Standard 7	1.744	1.634	74.6	78.125	1.500	1.394	74.1	39.06
Standard 8	1.935	1.825	83.4	39.06	1.675	1.569	83.5	19.53
B0	2.299	2.189	100	0	1.986	1.880	100	0
Sample 1	0.857	0.747	34.1	698.5	0.535	0.429	22.8	721.6
Sample 2	1.588	1.478	67.5	115.3	1.097	0.991	52.7	114.3

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

Conversion Factor: 1,000 ng/mL of human RBP is equivalent to 47.62 nM RBP.

*The MyAssays logo is a registered trademark of MyAssays Ltd.

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 #8. The sensitivity was determined at two (2) standard deviations from the B0 along the standard curve.

10 µL Format Sensitivity was determined as 7.06 ng/mL.
25 µL Format Sensitivity was determined as 2.93 ng/mL.

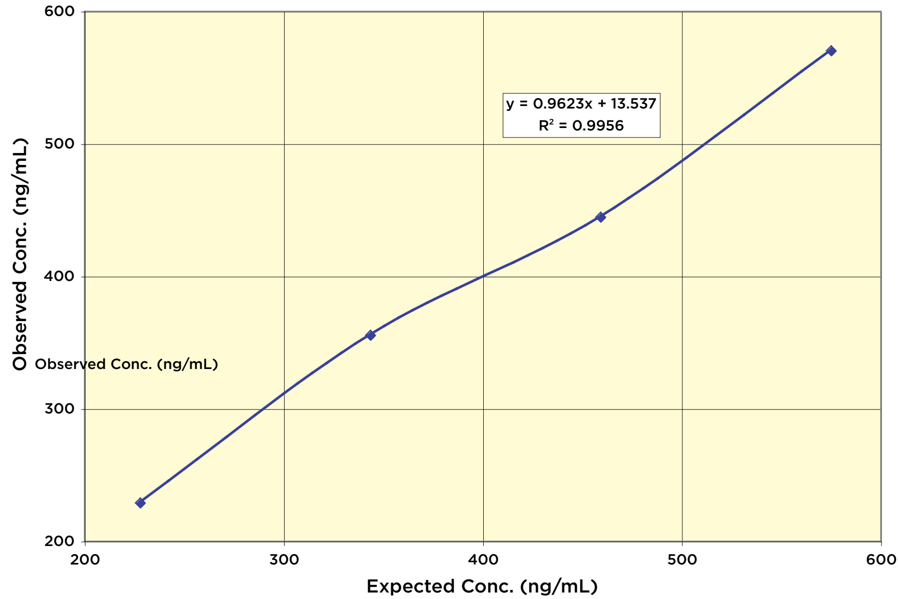
The Limit of Detection was determined in a similar manner by comparing the OD's for twenty wells for each of the zero standard and a low concentration human sample.

10 µL Format Limit of Detection was determined as 15.7 ng/mL.
25 µL Format Limit of Detection was determined as 5.26 ng/mL.

Linearity

Linearity was determined in the 10 µL format using human serum and EDTA plasma samples, by taking samples with a high known RBP concentration and a lower RBP concentration and mixing them in the ratios given below. The measured RBP 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
80%	20%	575.0	570.3	99.2
60%	40%	459.3	444.8	96.8
40%	60%	343.6	355.6	103.5
20%	80%	228.0	228.9	100.4
			Mean Recovery	**100.0%**



Intra Assay Precision - 10 µL sample

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

Sample	RBP Conc. (ng/mL)	%CV
1	614.2	5.6
2	227.1	8.2
3	90.8	12.9

Inter Assay Precision - 10 µL sample

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

Sample	RBP Conc. (ng/mL)	%CV
1	681.0	6.7
2	261.1	8.0
3	112.1	9.7

SAMPLE VALUES

Sixteen random human serum and plasma samples were tested in the assay. Values ranged from 23.2 to 35.2 µg/mL with an average of 29.6 µg/mL. The normal reference range for serum RBP is 30-60 µg/mL[10]. Samples with a serum RBP level below about 15 µg/mL RBP may be considered Vitamin A deficient[11].

Five random human whole blood DBS samples were punched out as 1/4” or 1/8” and tested in the assay. Values ranged from 7.54 to 19.7 µg/mL.

CROSS REACTIVITY AND INTERFERENTS

Two serum samples were spiked with varying concentrations of bilirubin, diluted 1:50 in Assay Buffer and tested in the assay. Bilirubin levels in normal serum are between 0.2 and 1.0 mg/dL[12]. No significant change to the measured RBP levels were observed up to an additional 10.0 mg/dL of bilirubin.

A serum sample was spiked with varying concentrations of hemoglobin, diluted 1:50 in Assay Buffer and tested in the assay. No significant change to the measured RBP level was observed up to an additional 2 mg/mL of hemoglobin. However, moderately to severely hemolyzed samples should be avoided as they have been shown to yield artificially high RBP concentrations.

A serum sample was spiked with varying concentrations of lipids, diluted 1:40 in Assay Buffer and tested in the assay. No significant change to the measured RBP level was observed with the addition of high, medium and low levels of lipids.

10. ARUP’s Laboratory Test Directory. “Retinol Binding Protein.” 2008.
    Internet: http://www.aruplab.com/guides/ug/tests/0050467.jsp (23 May 2008).

11. Hix J, Martinez C, Buchanan I, Morgan J, Tam M, Shankar A. “Development of a rapid enzyme immunoassay for the detection of retinol-binding protein.” Am J Clin Nutr 2004;79:93-8.

12. Tietz, N.W., in “Textbook of Clinical Chemistry”, WB Saunders, 1986.

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:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

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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