ARBOR ASSAYS™

DetectX® Thromboxane B_2 (TXB_2)
ELISA Kit

1 Plate Kit – Catalog No. K092-H1
5 Plate Kit – Catalog No. K092-H5

Sample Types Tested:
Serum, EDTA Plasma, Heparin Plasma, Urine, 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

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SUPPLIED COMPONENTS & STORAGE

		K092-H1	K092-H5	Description
Goat anti-Rabbit Clear Coated 96-well Plate	Quantity	1	5	Strip well plates coated with goat anti-rabbit IgG
	Catalog No.	X016-1EA	X016-1EA	
Thromboxane B_2 Standard	Volume	125 µL	625 µL	Thromboxane B_2 at 25,000 pg/mL in stabilizing solution
	Catalog No.	C329-125UL	C329-625UL	
DetectX® Thromboxane B_2 Antibody	Volume	3 mL	13 mL	Rabbit polyclonal antibody specific for thromboxane B_2
	Catalog No.	C330-3ML	C330-13ML	
DetectX® Thromboxane B_2 Conjugate	Volume	3 mL	13 mL	Thromboxane B_2-peroxidase conjugate in stabilizing solution
	Catalog No.	C331-3ML	C331-13ML	
Assay Buffer Concentrate 5x	Volume	28 mL	55 mL	5X concentrate that must be diluted
	Catalog No.	X067-28ML	X067-55ML	
Wash Buffer Concentrate 20x	Volume	30 mL	125 mL	20X concentrate that must be diluted
	Catalog No.	X007-30ML	X007-125ML	
TMB Substrate	Volume	11 mL	55 mL	3,3',5,5'-Tetramethylbenzidine, a substrate for HRP
	Catalog No.	X019-11ML	X019-55ML	
Stop Solution	Volume	5 mL	25 mL	1M solution of hydrochloric acid CAUSTIC
	Catalog No.	X020-5ML	X020-25ML	
Plate Sealer	Quantity	1	5	-
	Catalog No.	X002-1EA	X002-1EA	

The unopened kit must be stored at -20˚C.

Once opened, the kit can be stored at 4˚C up to the expiration date on the kit label.

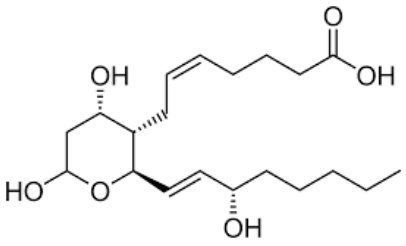
OTHER MATERIALS REQUIRED

- Distilled or deionized water.
- Adjustable pipettes with disposable tips capable of dispensing 25 µL, 50 µL, and 100 µL. Repeater pipette or multichannel pipettes with corresponding tips are also recommended.
- Glass or high-quality polypropylene test tubes for standard and sample preparation.
- An orbital microplate shaker.
- A plate reader capable of measuring absorbance at 450 nm.
- Software for converting optical density (OD) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.
- Optional: automated plate washer. Refer to Plate Washing Instructions for more details.
    - https://bit.ly/3tBT7N4
- Optional: Cyclooxygenase (COX) inhibitor such as indomethacin for serum and plasma samples.

PRECAUTIONS

- Read this insert completely prior to using the product.
- This kit may not perform as described if any reagent or procedure is replaced or modified. Do not interchange reagents from different kit lots.
- Take appropriate safety precautions, such as: avoid breathing fumes, wear personal protective equipment (gloves, clothing, eye, and face protection), and familiarize yourself with SDS documents.
    - https://www.ArborAssays.com/documentation/msds/K092-H_MSDS.pdf
- Ensure all buffers used for samples are azide free and that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer. Buffers, including other manufacturers' wash buffers, that contain sodium azide will inhibit color production from the enzyme.
- **Take appropriate precautions when handling the Stop Solution, which is a caustic acid.**

BACKGROUND



Thromboxane B_2 (TXB_2), $C_{20}H_{34}O_6$, is a stable metabolite produced by Thromboxane A_2 (TXA_2) involved in platelet activation and aggregation. The eicosanoid TXA_2 is the predominant product of cyclooxygenase, specifically COX-1[1]. COX-1 catalyzes the first two steps of prostaglandins (PGs) biosynthesis[2]. PGs play the key role in generation of an inflammatory response and are primary targets for nonsteroidal anti-inflammatory drugs (NSAIDs)[3]. TXA_2 itself has prothrombotic properties and is a known vasoconstrictor. It is also thought to play a role in the pathogenesis of myocardial infarction, stroke, atherosclerosis, and bronchial asthma[4]. TXA_2 is extremely unstable, with a half-life of 30 seconds[5]. Therefore TXB_2, after it is hydrated from active TXA_2[6], is the ideal candidate for a stable metabolite biomarker to use for an abundance of conditions and measuring anti-platelet drug effectiveness.

ASSAY PRINCIPLE

The DetectX® Thromboxane B_2 (TXB_2) ELISA Kit quantitatively measures TXB_2 in serum, plasma, urine, and tissue culture media samples. The TXB_2 ELISA Kit is a competitive ELISA with a run time of 2.5 hours. Please read this complete kit insert for more information before performing this assay.

A TXB_2 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 a polyclonal antibody to capture rabbit antibodies. A TXB_2-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 TXB_2 to each well. As the TXB_2 concentration in the sample increases, the bound TXB_2-peroxidase conjugate decreases, causing a decrease in signal and vice versa.

After an incubation, the plate is washed and substrate is added. The substrate reacts with the bound TXB_2-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 TXB_2 in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

REAGENT PREPARATION

Except for the reagents listed below, all kit components are ready for use.

Reagent	Preparation	Stability
1X Assay Buffer	Warm 5X Assay Buffer to room temperature and mix thoroughly by inversion. Mix 1 volume 5X Assay Buffer Concentrate with 4 volumes deionized water.	1X Assay Buffer is stable for 3 months at 4°C
1X Wash Buffer	Warm 20X Wash Buffer to room temperature and mix thoroughly by inversion. Mix 1 volume 20X Wash Buffer Concentrate with 19 volumes deionized water.	1X Wash Buffer is stable for 3 months at room temperature

SAMPLE PREPARATION

For samples containing particulates, centrifuge prior to use. Upon collection, all samples should be frozen rapidly and stored at -80˚C until testing.

Sample Type	Procedure
Serum and Plasma	• Collect samples with care to avoid hemolysis†. • To avoid exogenous TXB_2 production, add a general cyclooxygenase inhibitor (e.g. indomethacin, 15 µM) immediately after sample collection. • Prepare a minimum 8-fold dilution by adding 50 µL sample to 350 µL 1X Assay Buffer. • Samples may require further dilution with 1X Assay Buffer to fall within the standard curve range.
Urine	• Prepare a minimum 8-fold dilution of sample by adding 50 µL urine to 350 µL 1X Assay Buffer. • Samples may require further dilution with 1X Assay Buffer to fall within the standard curve range. • Use our Urinary Creatinine Detection Kits (K002-H) to measure urine creatinine for normalization of TXB_2 in urine specimens.
Tissue Culture Media (TCM)	• This assay has been validated using RPMI-1640. Other types of TCM should be validated before use. • Samples should be diluted in TCM and read off a standard curve generated in the same TCM. • Prepare a minimum 2-fold sample dilution with 250 µL TCM in 250 µL 1X Assay Buffer. • Samples may require further dilution with TCM or 1X Assay Buffer to fall within the standard curve range.
Extracted Samples*	For samples that need to be concentrated or contain known interfering substances, a detailed extraction protocol can be found on the Resource page at ArborAssays.com/resources/#protocols or using this link.

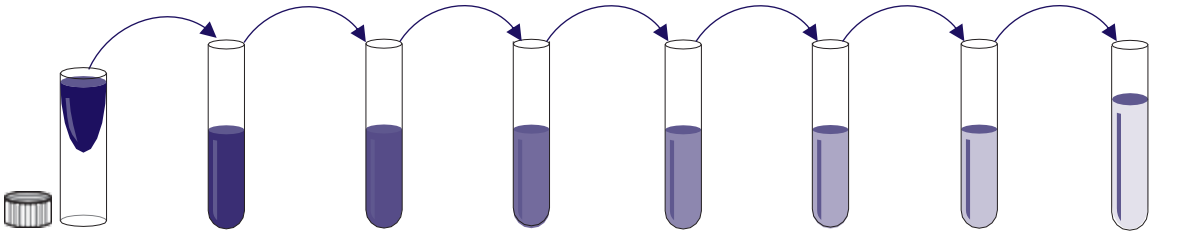
† Severely hemolyzed samples should not be used in this kit.

* Samples with high lipid content may interfere with the measurement of TXB_2 and may require extraction.

⚠ **Use all samples within 2 hours of dilution.**

STANDARD PREPARATION

1. Label tubes Standard 1 through Standard 7.
2. Add 450 µL 1X Assay Buffer to Standard 1 tube.
3. Add 260 µL 1X Assay Buffer to Standard 2 – 7 tubes.
4. Add 50 µL of the TXB_2 stock solution to Standard 1 tube. Vortex thoroughly.
   ⚠ The TXB_2 stock solution contains an organic solvent. Pipet the stock solution up and down several times prior to dispensing to ensure accurate delivery.
5. Transfer 200 µL of Standard 1 into Standard 2 tube to make a 2.3-fold dilution. Vortex thoroughly.
6. Transfer 200 µL of the mixed solution from Standard 2 into Standard 3 tube to make a 2.3-fold dilution. Vortex thoroughly.
7. Continue serially diluting into the remaining tubes. This process and the final concentrations are summarized in the table below.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
1X Assay Buffer (µL)	450	260	260	260	260	260	260
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	50	200	200	200	200	200	200
Final Concentration (pg/mL)	2,500	1,087	473	206	89.3	38.8	16.9

⚠ **Use all Standards within 2 hours of dilution.**

ASSAY PROTOCOL

Before You Begin:

- **Ensure all reagents have been warmed to room temperature.**
- **Dilute samples as described in Sample Preparation.**
- **Run all standards and samples in duplicate.**
- Use the blank plate template on the back page of this booklet to design your plate layout and aid in proper sample and standard identification.
- Be sure to shake the plate as directed. Failing to shake the plate or altering the shaking speed during incubations will result in decreased signal.
- Set plate parameters on the plate reader for a 96-well Corning CoStar 9017 plate. See ArborAssays.com for plate dimension data.
- Determine the number of strip wells to be used and return unused strip wells to the foil pouch with desiccant. Seal the foil pouch and store at 4°C. Desiccant color will change from blue to pink if the foil pouch is not properly sealed.
- If you are using only part of a strip well plate, at the end of the assay discard the used wells and retain the plate frame for use with the remaining unused wells.

1. Add 50 µL Samples or Standards into duplicate wells.
2. Add 75 µL 1X Assay Buffer into duplicate NSB (non-specific binding) wells.
3. Add 50 µL 1X Assay Buffer into duplicate Zero Standard (maximum binding or B0) wells.
4. Add 25 µL DetectX® Thromboxane B_2 Conjugate to each well using a repeater pipet.
5. Add 25 µL DetectX® Thromboxane B_2 Antibody to each well, **except the NSB wells**, using a repeater pipet.
6. Cover the plate with a plate sealer and shake at room temperature at 700-900 rpm for **2 hours**.
7. Remove the plate sealer, aspirate the plate, and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
8. Add 100 µL TMB Substrate to each well using a repeater pipet.
    - The substrate solution will begin to turn blue.
9. Incubate at room temperature for **30 minutes** without shaking.
10. Add 50 µL Stop Solution to each well using a repeater pipet.
    - The substrate solution will begin to turn yellow.
11. Read the optical density at 450 nm within 10 minutes.

CALCULATION OF RESULTS

Follow the instructions below, or use the online tool from MyAssays.
https://www.myassays.com/arbor-assays-detectx-thromboxane-b2.assay

1. Use four-parameter logistic curve (4PLC) software to calculate the TXB_2 concentration for each sample.
2. Gather all raw data OD readings from each Sample and Standard, including the Zero Standard (B0) and NSB.
3. Average the duplicate OD readings for each Sample, Standard, B0, and NSB (Mean OD).

EXAMPLE:

Sample	Replicate 1 OD	Replicate 2 OD	Mean OD
NSB	0.090	0.091	0.091
B0	1.420	1.376	1.398
Sample 1	0.648	0.715	0.682

4. Subtract the NSB from the Mean OD for each Sample, Standard, and the B0 (Net OD).

EXAMPLE:

Sample	Mean OD	NSB Mean OD	Net OD
B0	1.398	0.091	1.307
Sample 1	0.682	0.091	0.591

5. Divide the Net OD for each Sample and Standard by the Net OD for the B0 and multiply by 100% (%B/B0).

EXAMPLE:

Sample	Net OD	B0 Net OD	%B/B0
Sample 1	0.591	1.307	45.2

6. Plot the standard curve with %B/B0 for the Standards on the y-axis and TXB_2 concentration (pg/mL) on the x-axis. Perform a 4PLC fit.

   Use the sample %B/B0 readings and the 4PLC fit to calculate TXB_2 concentrations in diluted samples. If diluted sample cortisol concentrations are outside of the range of the standards, the sample should be prepared again at a more appropriate dilution.

EXAMPLE:

Sample	Net OD	%B/B0	Sample TXB_2 Concentration (pg/mL)
Sample 1	0.591	45.2	235

7. If the original sample was diluted, multiply the sample TXB_2 concentration by the sample dilution factor to determine the concentration of TXB_2 in the original sample.

EXAMPLE:

Sample	Sample TXB_2 Concentration (pg/mL)	Sample Dilution Factor	Original Sample TXB_2 Concentration (pg/mL)
Sample 1	235	8	1880

TYPICAL DATA

⚠ **Always run your own standard curve. This data should NOT be used to interpret experimental results.**

Sample	Mean OD	Net OD	%B/B0	Sample TXB_2 Concentration (pg/mL)
NSB	0.076	0.000	-	-
Standard 1	0.163	0.087	9.0	2,500
Standard 2	0.232	0.156	16.2	1,087
Standard 3	0.348	0.272	28.2	473
Standard 4	0.519	0.443	46.0	206
Standard 5	0.702	0.626	64.8	89.3
Standard 6	0.862	0.786	81.3	38.8
Standard 7	0.954	0.878	90.9	16.9
B0	1.050	0.974	100	0
Sample 1	0.369	0.293	30.1	429
Sample 2	0.789	0.713	73.0	61.1

Typical Standard Curve

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the B0 and Standard #7. The detection limit was determined at two standard deviations from the B0 along the standard curve.

Sensitivity was determined as 11.2 pg/mL.

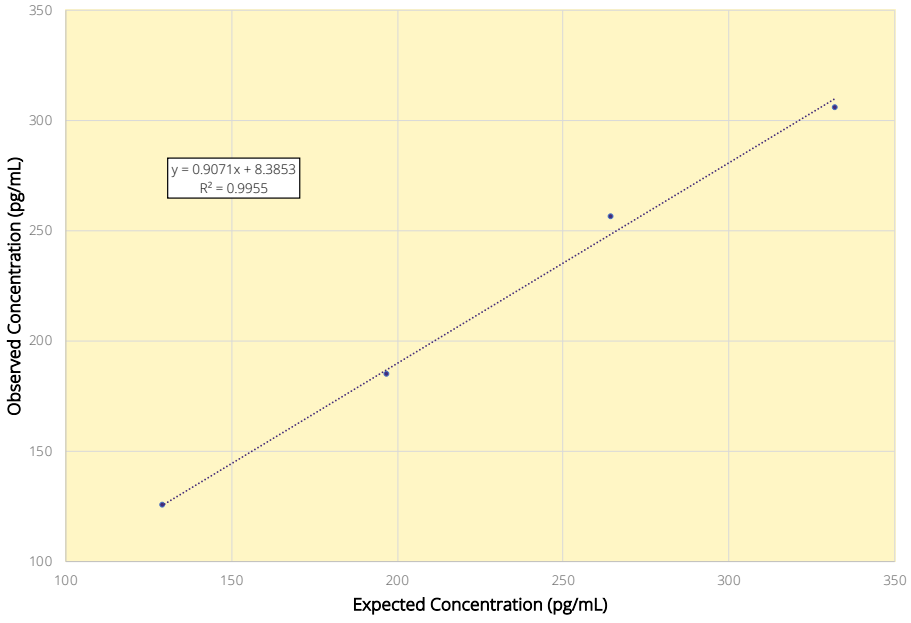
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the B0 and a low concentration sample.

The Limit of Detection was determined as 11.0 pg/mL.

Linearity

Linearity was determined in human serum by diluting two samples with known TXB_2 concentrations. One sample had a TXB_2 concentration of 62.3 pg/mL (low serum), and one had a TXB_2 level of 400 pg/mL (high serum). The two samples were mixed in the ratios given below, and the measured concentrations were compared to the expected values for each given ratio.

Low Serum	High Serum	Expected Concentration (pg/mL)	Observed Concentration (pg/mL)	% Recovery
80%	20%	130	125	96.6
60%	40%	197	185	93.6
40%	60%	265	256	96.8
20%	80%	332	306	92.0
			Mean Recovery	**94.7**



Intra Assay and Inter Assay Precision

For intra assay precision, three serum samples were diluted in 1X Assay Buffer and 22 replicates were run in one assay. For inter assay precision, three serum samples were diluted in 1X Assay Buffer and duplicates of each sample were run in twenty assays run over multiple days by multiple operators. %CV represents the variation in concentration (not optical density) as determined using a reference standard curve.

	Intra Assay Precision		Inter Assay Precision	
Sample	TXB_2 Concentration (pg/mL)	% CV	TXB_2 Concentration (pg/mL)	% CV
1	451	4.5	429	7.6
2	171	8.2	158	6.3
3	71.0	8.3	61.0	8.3

SAMPLE VALUES

7 human serum, 7 human plasma, and 4 human urine samples were diluted in 1X Assay Buffer and tested in the assay. The adjusted average concentration and sample range are shown below.

Sample Type	Recommended Minimum Dilution	Adjusted Average Concentration (pg/mL)	Adjusted Concentration (pg/mL) Range
Serum	1:8	3,267	411 - 9,747
Plasma	1:8	1,662	351 – 5,589
Urine	1:8	784	537 – 1,147
RPMI-1640	1:2	96.2%*	91.4 – 101.4%*

* Spiked recovery analysis was performed for RPMI-1640 tissue culture media.

INTERFERENCE

Potentially interfering substances were evaluated in the assay and the change in signal was calculated.

Interferent	Effect
DMSO (2.5%)	9.8% decrease
Ethanol (1.25%)	6.8% decrease
Methanol (10%)	4.0% decrease
SDS (0.6%)	33.8% decrease – Do Not Use
TritonX-100 (10%)	0.9% decrease
Tween-20 (0.6%)	33.9% decrease – Do Not Use
Hemoglobin (40 mg/dL)	4.1% increase
Bilirubin (5 mg/dL)	1.6% increase

CROSS REACTIVITY

The following cross reactants were tested in the assay at 40x, 4.0x, 0.4x and 0.04x concentration of the highest standard. Percent cross-reactivity was calculated comparing observed concentration to actual concentration of each cross reactant.

Eicosanoid	Cross Reactivity (%)
Thromboxane B_2	100
2,3-dinor Thromboxane B_2	35.1
Thromboxane B_3	20.9
11-dehydro Thromboxane B_2	2.2
Prostaglandin D_2	0.9
11-dehydro Thromboxane B_3	0.7
Prostaglandin I_2	< 0.01

TROUBLESHOOTING

Issue	Possible Cause & Solution
Reagent Shortage	• Check under the cap for additional reagent. Pulse spin reagent containers to collect contents prior to opening when possible. • When using a multichannel pipette, return unused reagent to container for later use.
Erratic Values	• Ensure the assay plate has been properly blotted after assay washes to remove residual wash buffer. • Prerinse pipet tips with desired reagent prior to aspirating the required volume. • Deliver volume with care to prevent splashing into adjacent wells.
High NSB	• Ensure assay plate has been properly washed with the number of washes indicated in the protocol. • Reagent contamination during assay setup. • Verify antibody was not added to the NSB wells.
Low Signal	• Confirm tools, equipment, reagents, and containers used do not contain any trace of sodium azide. • Altering shaking speeds or excluding shaking during incubation steps. • Verify the plate reader wavelength is 450 nm. • Confirm reagents are at room temperature prior to use.

CITATIONS

1. Szczuko, M., Kozioł, I., Kotlęga, D., Brodowski, J., & Drozd, A. (2021). The Role of Thromboxane in the Course and Treatment of Ischemic Stroke: Review. *International journal of molecular sciences*, *22*(21), 11644.
2. Rouzer, C. A., & Marnett, L. J. (2009). Cyclooxygenases: structural and functional insights. *Journal of lipid research*, *50 Suppl*(Suppl), S29–S34.
3. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011 May;31(5):986-1000.
4. Rucker D, Dhamoon AS. Physiology, Thromboxane A2. [Updated 2022 Sep 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-.
5. Li K, Zhao J, Wang M, Niu L, Wang Y, Li Y, Zheng Y. The Roles of Various Prostaglandins in Fibrosis: A Review. Biomolecules. 2021 May 24;11(6):789.
6. Catella, F., Healy, D., Lawson, J. A., & FitzGerald, G. A. (1986). 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(16), 5861–5865.

RELATED PRODUCTS

Kits	Catalog No.
PGE_2 Multi-Format ELISA Kits	K051-H1/H5
3',3'-Cyclic GAMP ELISA Kits	K073-H1/H5
2',3'-Cyclic GAMP ELISA Kits	K067-H1/H5
Arg8-Vasopressin (AVP) Colorimetric and Chemiluminescent ELISA Kits	K049-H1/H5 K049-C1/C5
Atrial Natriuretic Peptide (ANP) ELISA Kits	K071-H1/H5
B-type Natriuretic Peptide (BNP) Human ELISA Kit	K083-H1/H5
Cyclic GMP Direct ELISA Kits	K065-H1/H5
Endothelin-1 ELISA Kit	K045-H1
Nitric Oxide (NO) Colorimetric Detection Kit	K023-H1
ST2 Human ELISA Kit	K055-H1

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

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 assay kits and reagents for wildlife conservation research.

PLATE LAYOUT

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