Gentaur Europe BVBA
Voortstraat 49,
1910 Kampenhout
BELGIUM
Tel 0032 16 58 90 45
info@gentaur.com

ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Estrone
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K031-H1
5 Plate Kit Catalog Number K031-H5

Species Independent

Sample Types Validated:

Dried Fecal Extracts, Urine and Tissue Culture Media

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

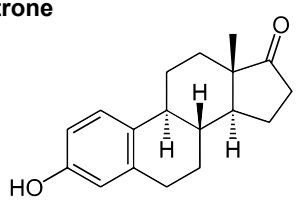
info@gentaur.com

TABLE OF CONTENTS

BACKGROUND

Estrone, $C_{18}H_{22}O_2$, also known as E1 or osterone (3-hydroxy-1,3,5(10)-estratrien-17-one) is a C-18 steroid hormone. Estrone is one of the three naturally occurring estrogens, the others being estradiol and estriol[1]. Estrone is produced primarily from androstenedione originating from the gonads or the adrenal cortex and from estradiol by 17-hydroxysteroid dehydrogenase[2]. Androstenedione is also converted into estrone by aromatase (CYP19) to estrone and is expressed in stromal and carcinoma or parenchymal components of breast cancer tissue[3]. Estrone concentrations in premenopausal mammals fluctuate according to the menstrual cycle. In premenopausal women, more than 50% of the estrone is secreted by the ovaries. In prepubertal children, men and non-supplemented postmenopausal women the major portion of estrone is derived from peripheral tissue conversion of androstenedione. Interconversion of estrone and estradiol also occurs in peripheral tissue. In humans, during the follicular phase of the menstrual cycle estrone levels increase slightly. The production of estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 the estrone levels will be low. A second peak occurs at around day 21 of the cycle and if fertilization does not occur, then the production of estrone decreases.

Estrone



1. Gruber, CJ, et. al. “Production and actions of estrogens.”, N. Engl. J. Med., 2002, 346:340-352.
2. Vance DE., “Cholesterol and related derivatives.” In: “Biochemistry”, G. Zubay, Ed., 1988, Macmillan Publishing Co., NY, NY, Pgs. 735-748.
3. Miki Y, et al. “Aromatase localization in human breast cancer tissues: possible interactions between intratumoral stromal and parenchymal cells.”, Cancer Res., 2007, 67:3945–3954.

ASSAY PRINCIPLE

The DetectX® Estrone Immunoassay Kit is designed to quantitatively measure estrone present in extracted dried fecal samples, urine and tissue culture media samples. The kit is unique as it measures both non-conjugated estrone and conjugated estrone, in the form of estrone-3-sulfate and estrone 3-glucuronide, in urine and fecal samples with almost equal affinity, allowing for non-invasive testing of this steroid.

Please read the complete kit insert before performing this assay. An estrone 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 an antibody to capture rabbit antibodies. An estrone-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 estrone to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound estrone-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 estrone 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.
17β-Estradiol Enzyme Immunoassay Kits	K030-H1/H5
Androstenedione ELISA Kits	K070-H1/H5
Corticosterone Enzyme Immunoassay Kits	K014-H1/H5
Cortisol Enzyme Immunoassay Kits (Strip Wells and Whole Plate)	K003-H1/H5/H1W/H5W
Cortisone ELISA and Chemiluminescent ELISA Kits	K017-H1/H5, K017-C1/C5
Epiandrosterone ELISA Kits	K063-H1/H5
Progesterone Metabolites ELISA Kits	K068-H1/H5
Urinary Creatinine Detection Kit (2 or 10 Plates)	K002-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
Clear, break-apart 1 by 8 strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG.

Kit K031-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA

Estrone Standard
Estrone at 20,000 pg/mL in a special stabilizing solution.

Kit K031-H1 **or** -H5	125 µL **or** 625 µL	Catalog Number C110-125UL **or** -625UL

DetectX® Estrone Antibody
A rabbit polyclonal antibody specific for estrone.

Kit K031-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C108-3ML **or** -13ML

DetectX® Estrone Conjugate Must be stored at -20°C.
A estrone-peroxidase conjugate in a special stabilizing solution.

Kit K031-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C109-3ML **or** -13ML

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

Kit K031-H1 **or** -H5	28 mL **or** 55 mL	Catalog Number X065-28ML **or** -55ML

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

Kit K031-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K031-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K031-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K031-H1 **or** -H5	1 **or** 5 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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, **except for the Estrone Conjugate. This must be stored at -20°C.**

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes.

Ethanol for extraction of fecal material.

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

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

A Speedvac/centrifugal concentrator or N_2 gas and gas manifold for evaporation.

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 dried fecal, urine and for tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Estrone can be assayed in other sample types by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols.

Estrone is identical across all species and we expect this kit to measure estrone from all sources. The end user should evaluate recoveries of estrone in other sample matrices being tested.

SAMPLE PREPARATION

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%.

Urine Samples

Urine samples should be diluted ≥ 1:8 with the diluted Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection Kits, K002-H1 and K002-H5.

Tissue Culture Media

For measuring estrone 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. Using RPMI-1640 media we obtained 103% agreement of standards in TCM read off the Assay Buffer standard curve.

Use all samples within 2 hours 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

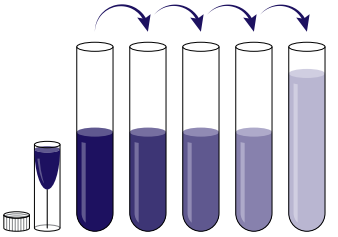
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 #7. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #7. **The estrone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 µL of the estrone stock solution to tube #1 and vortex completely. Take 250 µL of the estrone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of estrone in tubes 1 through 7 will be 2,000, 1,000, 500, 250, 125, 62.5, and 31.25 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	**450**	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**50**	250	250	250	250	250	250
Final Conc (pg/mL)	2,000	1,000	500	250	125	62.5	31.25

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine estrone 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® Estrone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Estrone 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 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken, OD signal will be approximately 24% lower. %B/B0 will not be affected.
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 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 estrone 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.

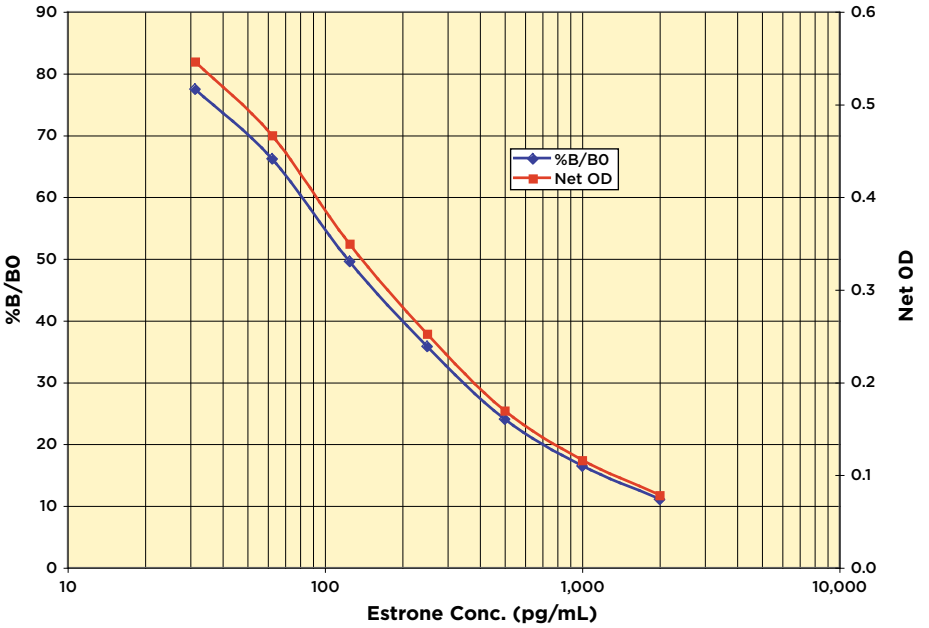
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-estrone-eia-kit.assay

TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Estrone Conc. (pg/mL)
NSB	0.087	0	-	-
Standard 1	0.165	0.078	11.1	2,000
Standard 2	0.203	0.116	16.5	1,000
Standard 3	0.256	0.170	24.0	500
Standard 4	0.339	0.253	35.8	250
Standard 5	0.436	0.350	49.6	125
Standard 6	0.553	0.467	66.2	62.5
Standard 7	0.633	0.546	77.4	31.25
B0	0.792	0.705	100	0
Sample 1	0.319	0.233	33.0	288.3
Sample 2	0.537	0.451	63.9	67.2

Always run your own standard curve for calculation of results. Do not use this data.
Conversion Factor: 100 pg/mL of estrone is equivalent to 369.9 pM.

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 #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 22.4 pg/mL.**

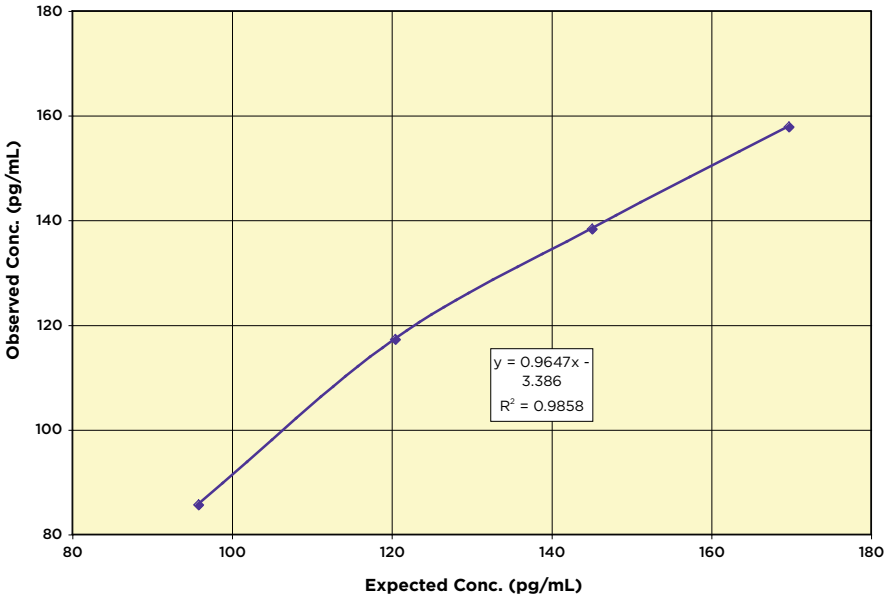
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample. **Limit of Detection was determined as 28.2 pg/mL.**

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted estrone level of 71.2 pg/mL and one with a higher diluted level of 194.4 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 Urine	Low Urine	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	169.8	157.8	93.0
60%	40%	145.1	138.3	95.3
40%	60%	120.5	117.2	97.3
20%	80%	95.8	85.6	89.3
			Mean Recovery	**93.7%**

Linearity



Intra Assay Precision

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

Sample	Estrone Conc. (pg/mL)	%CV
1	278.1	4.0
2	165.7	4.4
3	65.7	5.7

Inter Assay Precision

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

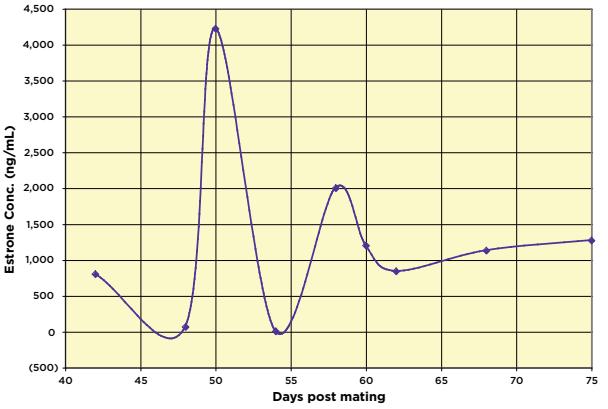
Sample	Estrone Conc. (pg/mL)	%CV
1	277.5	4.2
2	168.2	4.9
3	64.2	7.3

SAMPLE VALUES

Six human urine samples were tested in the assay. Adjusted neat concentrations of estrone ranged from 1.28 to 26.3 ng/mL, with an average value of 8.94 ng/mL. When adjusted for urine creatinine using the DetectX® Urinary Creatinine Detection Kit, K002-H1, the values ranged from 6.4 to 24.9 ng/mg creatinine, with an average value of 13.5 ng/mg creatinine.

Timed urine samples from a pregnant Maned Wolf over a 33-day period were tested in the assay. Day 75 was post birth.

Maned Wolf Urine



Maned wolf samples were the kind gift of Dr. Rachel Santymire from Lincoln Park Zoo, Chicago.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Estrone	100%
Estrone 3-glucuronide	112%
Estrone 3-sulfate	65.5%
Estradiol	5.0%
Estradiol-3-sulfate	<0.1%
Estriol	<0.1%
Progesterone	<0.1%
Pregnandiol	<0.1%
Cortisol	< 0.1%
Androsterone	< 0.1%

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

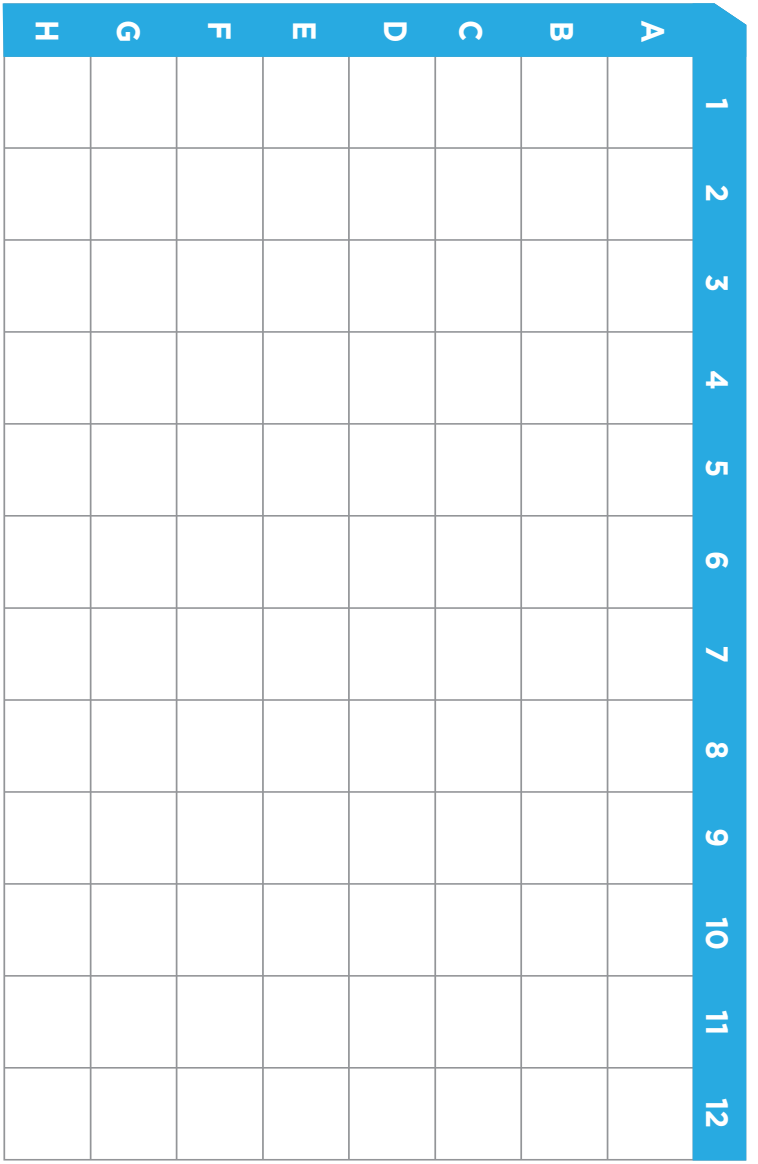
Technical@ArborAssays.com

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 ELISA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE



Printed on Forest Stewardship Council certified paper