ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
N-CAL™ KIT

NCal™ International Standard Kit

DetectX®

Prolactin

Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K040-H1

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.
Not for human diagnostic use.

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BACKGROUND

Prolactin (PRL) is a polypeptide hormone that is synthesized and secreted from specialized cells of the anterior pituitary gland. The hormone was given its name based on the fact that an extract of bovine pituitary gland would cause growth of the crop sac and stimulate the production of milk in pigeons or promote lactation in rabbits[1]. However it is now appreciated that prolactin has over 300 separate biological activities[2]. Prolactin has multiple roles in reproduction other than lactation, and it also plays multiple homeostatic roles in the organism. Furthermore, the synthesis and secretion of prolactin is not restricted to the anterior pituitary gland, but multiple other organs and tissues in the body have this capability.

The prolactin gene is composed of 5 exons and 4 introns[3, 4]. Transcription of the prolactin gene is regulated by two independent promoter regions. The human prolactin cDNA is 914 nucleotides long and generates the prolactin prohormone of 227 amino acids. After cleavage of the 28 amino acid signal peptide, mature human prolactin is composed of 199 amino acids[5]. The prolactin molecule is arranged in a single chain of amino acids with three intramolecular disulfide bonds between six cysteine residues (Cys^{4}-Cys^{11}, Cys^{58}-Cys^{174}, and Cys^{191}-Cys^{199} in humans)[3]. The sequence homology can vary from up to 97% among primates to as low as 56% between primates and rodents[5]. In rats[6] and mice[7], pituitary prolactin consists of 197 amino acids, whereas in sheep, pigs, cattle, and humans[8] it consists of 199 amino acids with a molecular weight of about 23,000 Da.

Prolactin is synthesized by the anterior pituitary, placenta, brain, uterus, dermal fibroblasts, decidua, B cell, T cells, NK cells, and breast cancer cells. Originally characterized as a lactogenic hormone, studies have demonstrated broader roles in breast cancer development, regulation of reproductive function, and immuno-regulation. Various cells have been shown to express the prolactin receptor. Three forms of the receptor, generated by differential splicing, have been identified. These isoforms differ in the length of their cytoplasmic domains. It is believed that the short cytoplasmic form is nonfunctional. Prolactin signal transduction involves the JAK/STAT families and Src kinase family.

1. Riddle O, Bates RW, and Dykshorn SW. The preparation, identification and assay of prolactin—a hormone of anterior pituitary. Am J Physiol (1933) 105:191–216.
2. Bole-Feysot C, Goffin V, Edery M, Binart N, and Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev. 1998 19:225–268.
3. Cooke NE, Coit D, Shine J, Baxter JD, and Martial JA. Human prolactin cDNA structural analysis and evolutionary comparisons. J. Biol. Chem. 1981, 256: 4007–4016.
4. Truong AT, Duez C, Belayew A, Renard A, Pictet R, Bell GI, and Martial JA. Isolation and characterization of the human prolactin gene. EMBO J., 1984, 3:429–437.
5. Sinha YN. Structural variants of prolactin: occurrence and physiological significance. Endocr. Rev., 1995, 16:354–369.
6. Cooke NE, Coit D, Weiner RI, Baxter JD, and Martial JA. Structure of cloned DNA complementary to rat prolactin messenger RNA. J. Biol. Chem. 1980, 255:6502–6510.
7. Kohmoto K, Tsunasawa S, AND Sakiyama F. Complete amino acid sequence of mouse prolactin. Eur. J. Biochem. 1984, 138:227–237.
8. Li CH, et. al.. Biochem. Biophys., 1970, 141:705.; Li CH., Int. J. Pept. Protein Res 1976, 8:205; Wallis M., FEBS Lett. 1974, 44:205; Shome B and Parlow AF., J. Clin. Endo. Metab. 1977, 45:1112.

ASSAY PRINCIPLE

The DetectX® Prolactin Immunoassay Kit is designed to quantitatively measure prolactin present in biological samples and tissue culture media. The kit has been tested to measure human and elephant serum prolactin. Please read the complete kit insert before performing this assay. A human prolactin 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 monoclonal antibody to capture the prolactin present. After a 60 minute incubation, the plate is washed and a peroxidase conjugated prolactin polyclonal antibody is added. The plate is again incubated for 60 minutes and washed. Substrate is then added to the plate, which reacts with the bound prolactin antibody conjugate. After a third 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 prolactin in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
17-Hydroxyprogesterone ELISA Kits	K053-H1/H5
Aldosterone ELISA & Chemiluminescent ELISA Kits	K052-H1/H5, K052-C1/C5
Allopregnanolone ELISA Kit, Monoclonal Antibody Based	K061-H1/H5
Androstenedione ELISA Kits	K070-H1/H5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Dehydro-epiandrosterone sulfate (DHEA-S) ELISA Kits	K054-H1/H5
Estradiol Non-Invasive & Serum ELISA Kits	K030-H1/H5, KB30-H1/H5
Estrone ELISA Kits	K031-H1/H5
Estrone-3-Glucuronide (E1G) ELISA Kits	K036-H1/H5
Hemoglobin Colorimetric Detection Kit	K013-H1
Hemoglobin High Sensitivity Detection Kits	K013-HX1/HX5
Levonorgestrel ELISA Kits	K058-H1/H5
Oxytocin ELISA & Chemiluminescent ELISA Kits	K048-H1/H5
PGFM (13,14-dihydro-15-keto-Prostaglandin F2alpha) ELISA Kits	K022-H1/H5
Pregnanediol 3-Glucuronide (PDG) ELISA Kits	K037-H1/H5
Progesterone ELISA Kits	K025-H1/H5
Progesterone Metabolites ELISA Kits	K068-H1/H5
Testosterone ELISA Kits	K032-H1/H5

SUPPLIED COMPONENTS

Clear Coated 96 Well Plate
Clear plastic microplate with break-apart strips coated with mouse anti-Prolactin.
One Plate — Catalog Number C140-1EA

Prolactin Standard
Two vials of recombinant human Prolactin at 500 pg. **Must be stored at -20°C.**
2 Vials — Catalog Number C139-1EA

DetectX® Prolactin Conjugate
A polyclonal antibody to Prolactin labeled with peroxidase. **Must be stored at -20°C.**
5 mL — Catalog Number C138-5ML

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

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
2 each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C.Once opened, the kit, **except the Prolactin standard and Conjugate**, should then be stored at 4 °C until the expiration date of the kit.

The Prolactin Standard and Conjugate MUST be stored at -20°C.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes.

Repeater pipet and disposable tips capable of dispensing 100 µL and 50 µL.

A microplate 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. 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 human serum, plasma, and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using.

This assay has been used to successfully measure human, seal and elephant (*Loxodonta africana and Elephas maximus*) Prolactin. Felids are reported NOT to be detected with this assay. Other species have not been evaluated in this kit.

SAMPLE PREPARATION

Serum and Plasma

Serum and plasma samples must be diluted ≥ 1:4 with the diluted Assay Buffer prior to running in the kit. Most normal human serum or plasma samples will require a dilution of ≥ 1:10 to allow detection within the standard curve and samples from pregnant humans may have to be diluted up to 1:100.

The kit uses a human Prolactin standard and values expressed for other non-human species will be expressed as pg/mL human Prolactin.

TCM

TCM samples should be diluted in TCM and read off a standard curve generated in the same TCM or diluted ≥ 1:20 in Assay Buffer and read off a standard curve generated in Assay Buffer.

Any samples with prolactin concentrations outside the standard curve range should be diluted further with Assay Buffer or TCM, as appropriate, to obtain readings within the standard curve range.

Use all samples within 2 hours of dilution.

It is up to the end user to determine the appropriate dilution for their samples.

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

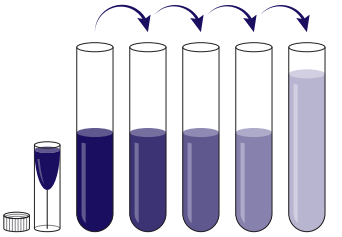
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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

Add 500 µL of Assay Buffer to one vial of Prolactin standard to generate the 1,000 pg/mL Standard 1 and mix well. Label test tubes as #2 through #7. Pipet 150 µL of Assay Buffer into tubes #2 to #7. Add 150 µL of the 1,000 pg/mL Prolactin standard to tube #2 and vortex completely. Take 150 µL of the Prolactin solution in tube #2 and add it to tube #3 and vortex completely. Repeat the serial dilutions for tubes #4 through #7. The concentration of Prolactin in the vial and tubes #2 through #7 will be 1,000, 500, 250, 125, 62.5, 31.25 and 15.625 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 Volume (µL)	**500**	150	150	150	150	150	150
Addition	**Vial**	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	**-**	150	150	150	150	150	150
Final Conc (pg/mL)	**1,000**	500	250	125	62.5	31.25	15.625

ASSAY PROTOCOL

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

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to 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. Pipet 50 µL of Assay Buffer into the zero standard wells.
3. Cover the plate with the plate sealer and shake at room temperature for 60 minutes. We recommend shaking at around 700–900 rpm.
4. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
5. Add 50 µL of the DetectX® Prolactin Conjugate to each well, using a repeater pipet.
6. Cover the plate with the plate sealer and shake at room temperature for 60 minutes. We recommend shaking at around 700–900 rpm.
7. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
8. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
9. Incubate the plate at room temperature for 30 minutes without shaking.
10. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
11. Read the optical density generated from each well at 450 nm.
12. Use the plate reader's built-in 4PLC software capabilities to calculate prolactin 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. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-prolactin-detection-kit-k040.assay

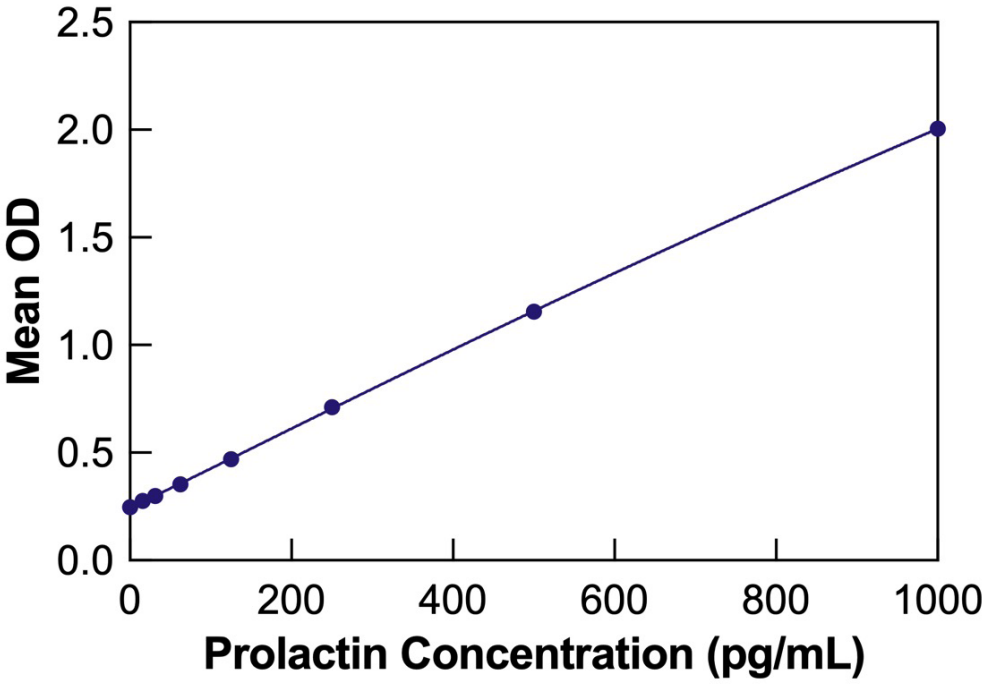
TYPICAL DATA

Sample	Mean OD	Prolactin Conc. (pg/mL)
Standard 1	2.005	1,000
Standard 2	1.155	500
Standard 3	0.711	250
Standard 4	0.469	125
Standard 5	0.354	62.5
Standard 6	0.299	31.25
Standard 7	0.276	15.625
Zero	0.246	0
Sample 1	1.115	475.68
Sample 2	0.841	324.53

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

1 ng of human Prolactin is equivalent to 42.94 microunits (µU) of the WHO 3rd International Standard (coded 84/500)

Typical Standard Curve



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

VALIDATION DATA

Sensitivity and Limit of Detection

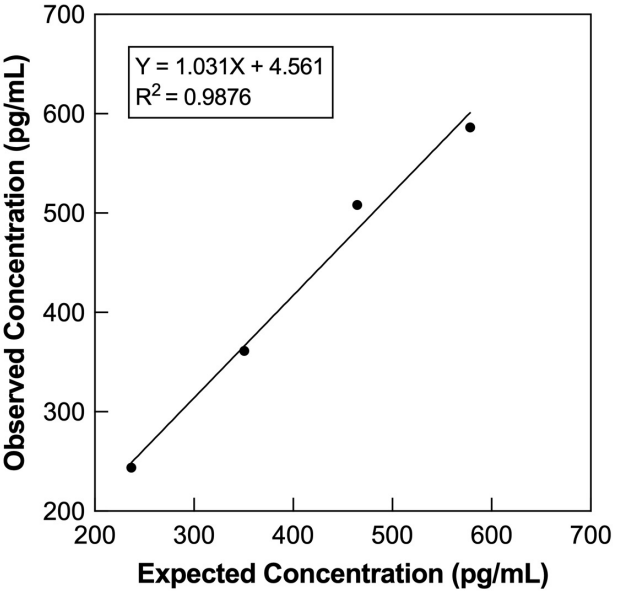
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 11.7 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration human urine sample.**Limit of Detection was determined as 13.4 pg/mL.**

Linearity

Linearity was determined by taking two human serum samples diluted 1:60, one with a low diluted Prolactin level of 122.9 pg/mL and one with a higher diluted level of 692.3 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the values previously determined.

High Sample	Low sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	578.4	586.3	101.4
60%	40%	464.5	508.1	109.4
40%	60%	350.6	361.3	103.0
20%	80%	236.8	243.8	103.0
			Mean Recovery	**104.2%**



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 Prolactin concentrations were:

Sample	Prolactin Conc. (pg/mL)	%CV
1	883.8	2.9
2	290.7	5.7
3	73.1	14.3

Inter Assay Precision

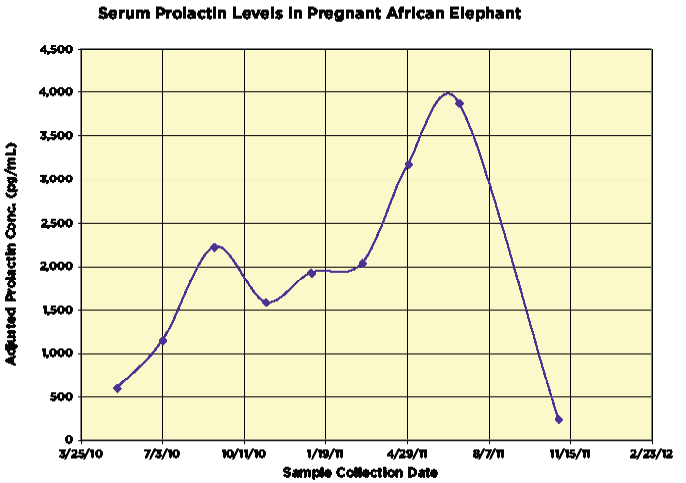
Three human samples were diluted with Assay Buffer and run in duplicates in fourteen assays run over multiple days by three operators. The mean and precision of the calculated Prolactin concentrations were:

Sample	Prolactin Conc. (pg/mL)	%CV
1	871.6	3.9
2	290.4	8.2
3	59.6	20.2

SAMPLE VALUES

A number of human female non-pregnant serum samples were tested in the kit after dilution from 20 to 80 fold. Normal non-pregnant human serum sample adjusted prolactin concentrations ranged from 3.55 to 9.24 ng/mL. Normal ranges for human prolactin range from 3-27 ng/mL. Five serum samples from pregnant females were also run after dilution from 1:40 to 1:640, yielding adjusted prolactin concentrations ranged from 16.5 to 43.2 ng/mL. Prolactin levels typically rise 10-20 fold in pregnancy.

Five elephant serum samples were tested with this assay. Four were from African elephants and one from an Asian elephant. Non-pregnant African elephant samples read between 97 and 220 pg/mL (adjusted), whereas serum from a pregnant elephant had levels that ranged from 244 to almost 4,000 pg/mL (adjusted) during the term of the pregnancy. See time course below.



CROSS REACTIVITY

This kit was developed to measure prolactin in elephant serum and has been tested in both African and Asian elephant sera.

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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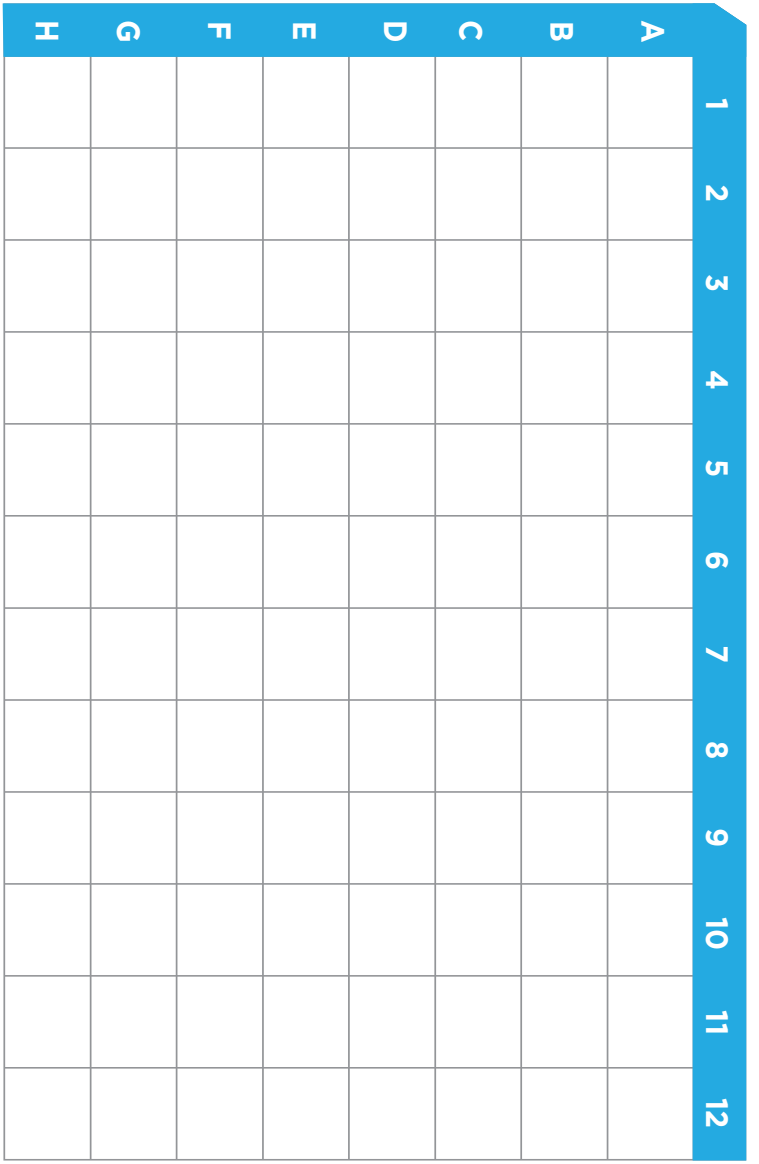
OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with ELISA kits for wildlife conservation research.



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