ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Cortisone

Chemiluminescent Immunoassay Kit

1 Plate Kit Catalog Number K017-C1

5 Plate Kit Catalog Number K017-C5

Species Independent

Improved Standard Range - New Sample Volume

Sample Types Validated:

Dried Fecal Extracts, Urine, Saliva, Plasma, Serum and 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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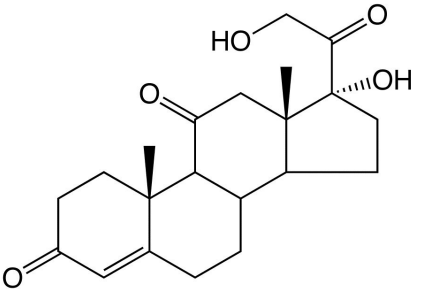
BACKGROUND

Cortisone ($C_{21}H_{28}O_5$, Kendall's Compound 'E') was identified by Mason, Myers and Kendall in 1936 as Compound E extracted from bovine suprarenal gland tissue that had the qualitative but not quantitative activity of cortin. The presence of multiple cortin-like compounds led the authors to speculate that the study of Compound E would reveal the nature of cortin[1]. Compound E is now called cortisone and the more active Compound F, cortisol, and the concentrations of these two glucocorticoids vary due to the activity of two 11β-hydroxysteroid dehydrogenases (11β-HSD) [2,3]. While most tissues have the ability to express either enzyme, 11β-HSD1 is found primarily in the liver where it converts cortisone to cortisol while 11β-HSD2 is found in tissues such as the kidney where cortisol receptor binding is required. 11β-HSD2 deactivates cortisol to cortisone, prohibiting receptor activation. This glucocorticoid "shuttle" helps to initiate and regulate the anti-inflammatory response, making cortisone one of the modern "wonder drugs". Monitoring the ratio of cortisone:cortisol has applications in diabetes, obesity, metabolic syndrome, osteoporosis, and chronic fatigue syndrome in addition to adrenal diseases[4-7]. Cortisone and cortisol concentrations exhibit a predictable diurnal pattern and can be measured in extracted dried feces, or in serum, plasma, saliva and urine. A 2010 publication[8] has suggested that salivary cortisone is a good surrogate marker for serum cortisol.



1. Mason, HL, et al., "Chemical Studies of the Suprarenal Cortex: II. The Identification of a Substance Which Possess the Qualitative Action of Cortin; Its Conversion into a Diketone Closely Related to Androstenedione" J. Biol. Chem., 1936 116:267-276.
2. Mason, HL, et. al., "Chemical Studies of the Suprarenal Cortex: IV. Structures of Compounds C, D, E, F, and G" J. Biol. Chem., 1938 124:459-474.
3. Hillier, SG. "Diamonds are Forever: the Cortisone Legacy" J. Endo., 2007 195:1-6.
4. van Raalte, DH, et al., "Novel Insights into Glucocorticoid-mediated Diabetogenic Effects: Towards Expansion of Therapeutic Options?" Eur. J. Clin. Invest. 2009 39(2):81-93.
5. Pierotti, S, et al., "Pre-receptorial Regulation of Steroid Hormones in Bond Cells: Insights on Glucocorticoid-induced Osteoporosis" J. Steroid Biochem. Mol. Biol. 2008 108(3-5):292-9.
6. Hadoke, PWF, et al., "Therapeutic Manipulation of Glucocorticoid Metabolism in Cardiovascular Disease" Br. J. Pharmacol. 2009 156:689-712.
7. Jerkes, WK, et al., "Diurnal Excretion of Urinary Cortisol, Cortisone, and Cortisol Metabolites in Chromic Fatigue Syndrome" J. Psychosomatic Res. 2006 60:145-153.
8. Perogamvros, I, et al., "Salivary Cortisone is a Potential Biomarker for Serum Free Cortisol" J Clin. Endocrin. Metab. 2010 August 4 (Epub ahead of print).

ASSAY PRINCIPLE

The DetectX® Cortisone Chemiluminescent Immunoassay Kit is designed to quantitatively measure Cortisone present in extracted dried fecal samples, urine, saliva, plasma, and serum samples. Please read the complete kit insert before performing this assay. This kit measures total cortisone in serum and plasma and in extracted samples. A cortisone 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. A cortisone-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 cortisone to each well. After a two hour incubation the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound cortisone-peroxidase conjugate to produce light. The generated light is detected in a microtiter plate reader capable of reading luminescence. The concentration of the cortisone 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.
Corticosterone Chemiluminescent ELISA Kits	K014-C1/C5
Corticosterone ELISA Kits	K014-H1/H5
Cortisol ELISA Kits	K003-H1/H5
Cortisone ELISA Kits	K017-H1/H5

SUPPLIED COMPONENTS

Coated White 96 Well Plates
A white plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG.

Kit K017-C1 **or** -C5	1 **or** 5 Each	Catalog Number X014-1EA

Cortisone Standard
Cortisone at 1,000 ng/mL in a special stabilizing solution.

Kit K017-C1 **or** -C5	50 µL **or** 125 µL	Catalog Number C054-50UL **or** -125UL

DetectX® Cortisone CLIA Antibody
A rabbit polyclonal antibody specific for cortisone.

Kit K017-C1 **or** -C5	3 mL **or** 13 mL	Catalog Number C055-3ML **or** -13ML

DetectX® Cortisone CLIA Conjugate Concentrate
A cortisone-peroxidase conjugate concentrate in a special stabilizing solution.

Kit K017-C1 **or** -C5	1 mL **or** 3.5 mL	Catalog Number C056-1ML **or** -3.5ML

Conjugate Diluent
Contains special stabilizers and additives.

Kit K017-C1 **or** -C5	3 mL **or** 13 mL	Catalog Number X076-3ML **or** -13ML

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

Kit K017-C1 **or** -C5	28 mL **or** 55 mL	Catalog Number X088-28ML **or** -55ML

Dissociation Reagent
NOTE: Dissociation Reagent is to be used only with Serum samples.

Kit K017-C1 **or** -C5	1 mL **or** 5 mL	Catalog Number X058-1ML **or** -5ML

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

Kit K017-C1 **or** -C5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

Substrate Solution A

Kit K017-C1 **or** -C5	6 mL **or** 28 mL	Catalog Number X077-6ML **or** -28ML

Substrate Solution B

Kit K017-C1 **or** -C5	6 mL **or** 28 mL	Catalog Number X078-6ML **or** -28ML

Plate Sealer

Kit K017-C1 **or** -C5	1 **or** 5 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 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 dependeant 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 diluted Cortisone CLIA Conjugate as prepared on page 8 into 995 µL of deionized water. Pipet 5 µL of diluted conjugate into a white well and add 100 µL of prepared CLIA substrate (see page 8 for details). This well will give you an intensity close to the maximum binding signal for the assay. Adjust the gain, integration time, or sensitivity so that your reader is giving close to its 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 urine, saliva, plasma and serum samples, and cell cultire media. It has also been validated for dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit.

Cortisone is identical across all species and we expect this kit should measure cortisone from sources other than human. The end user should evaluate recoveries of cortisone in other samples being tested.

SAMPLE PREPARATION

Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total cortisone concentration in serum. **Dissociation Reagent is to be used only with Serum or Plasma samples.** Free cortisone can be measured in saliva and urine samples as directed below.

Dried Fecal Samples

Dried fecal samples must be extracted. Detailed extraction protocols are available online: www.ArborAssays.com/resources/#protocols. The ethanol concentration in the final Assay Buffer dilution added to the well should be < 5%.

Saliva Samples

Saliva samples should be frozen and thawed, then centrifuged at 14,000 rpm for 15 minutes. The supernatant should be diluted ≥ 1:5 with the supplied Assay Buffer prior to running in the assay.

See our Saliva Sample Handling Instructions at: www.ArborAssays.com/assets/saliva-sample-protocol.pdf

Urine Samples

Urine samples should be diluted ≥ 1:100 with the supplied Assay Buffer prior to running in the assay.

Serum and Plasma Samples

Allow the Dissociation Reagent (DR) to warm completely to **Room Temperature** before use. We suggest pipeting 5 µL of DR into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the DR in the tube, vortex gently, and incubate at room temperature for 5 minutes or longer. Dilute with 490 µL of supplied Assay Buffer. This 1:100 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be ≥ 1:100.

NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

Tissue Culture Media

For measuring cortisone 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. We have validated the assay using RPMI-1640.

Use all Samples within 2 Hours of preparation, or store at -20°C until assaying.

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

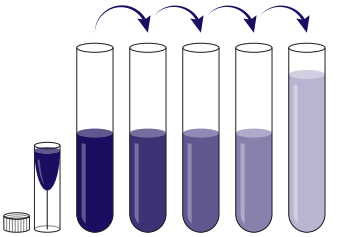
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Cortisone Conjugate

The supplied Cortisone Conjugate Concentrate should be diluted 1:4 with the Conjugate Diluent. Once diluted the diluted Cortisone Conjugate is stable at 4°C for one month.

Standard Preparation

Label test tubes as #1 through #6. Pipet 490 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #6. Carefully add 10 µL of the cortisone stock solution to tube #1 and vortex completely. Take 100 µL of the cortisone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of cortisone in tubes 1 through 6 will be 20,000, 5,000, 1,250, 312.5, 78.13, and 19.53 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**490**	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**10**	100	100	100	100	100
Final Conc (pg/mL)	20,000	5,000	1,250	312.5	78.13	19.53

Chemiluminescent Substrate

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

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Cortisone 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 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® Cortisone Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® Cortisone 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 signals bound will be approximately 45% lower.
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 mixed Chemiluminescent Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 5 minutes without shaking.
11. Read the luminescence generated from each well in a multimode or chemiluminescent plate reader using a 0.1 second read time per well.
12. The chemiluminescent signal will decrease about 40% over 60 minutes.
13. Use the plate reader's built-in 4PLC software capabilities to calculate Cortisone concentration for each sample.

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

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. Subtract the mean RLUs for the NSB from the mean RLUs of all the standards and samples and create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

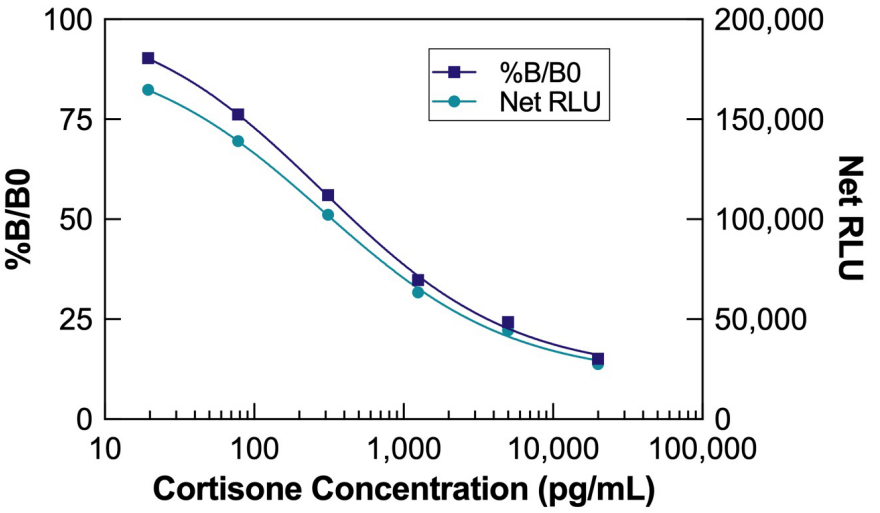
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-cortisone-chemiluminescent-clia-kit.assay

TYPICAL DATA

Sample	Mean RLU	Net RLU	% B/B0	Cortisone Conc. (pg/mL)
NSB	8,860	0		-
Standard 1	36,525	27,665	15.16	20,000
Standard 2	53,240	44,380	24.32	5,000
Standard 3	72,325	63,465	34.77	1,250
Standard 4	111,150	102,290	56.05	312.5
Standard 5	147,930	139,070	76.20	78.13
Standard 6	173,570	164,710	90.25	19.53
B0	191,365	182,505	100	0
Sample 1	102,960	94,100	51.56	421.9
Sample 2	141,395	132,535	72.62	101.4

Always run your own standard curve for calculation of results. Do not use this data.
Conversion Factor: 100 pg/mL of Cortisone is equivalent to 277.6 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

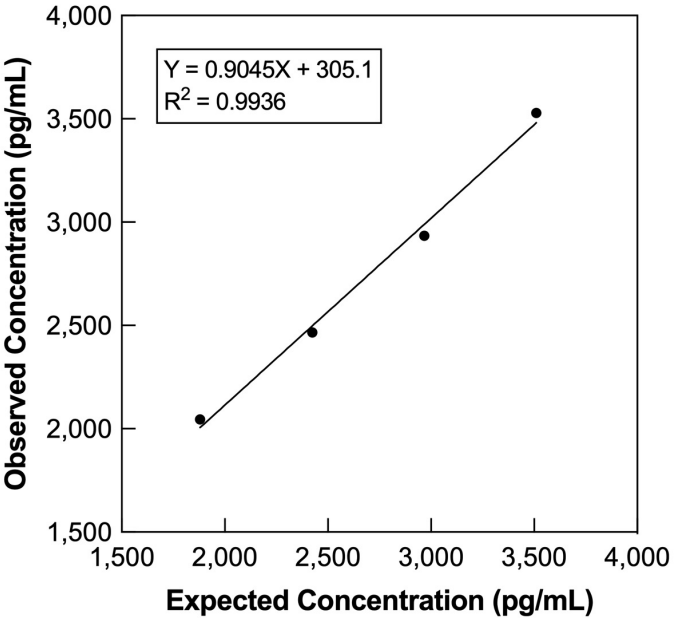
Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 10.6 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of the zero standard and a low concentration human sample. **Limit of Detection was determined as 16.2 pg/mL**

Linearity

Linearity was determined by taking two serum samples treated with Dissociation Reagent and further diluted with Assay Buffer, one with a low diluted cortisone level of 1,336 pg/mL and one with a higher level of 4,055 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Serum	High Serum	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	1,880	2,045	108.8
60%	40%	2,424	2,466	101.7
40%	60%	2,967	2,934	98.9
20%	80%	3,511	3,528	100.5
			Mean Recovery	**102.5%**



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 cortisone concentrations were:

Sample	Cortisone Conc. (pg/mL)	%CV
1	116.4	13.0
2	414.6	8.6
3	3,320	10.8

Inter Assay Precision

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

Sample	Cortisone Conc. (pg/mL)	%CV
1	108.1	15.1
2	414.5	9.0
3	3,183	9.8

SAMPLE VALUES

Nineteen random human serum samples were tested in the assay. Neat sample values ranged from 8.8 to 63.3 ng/mL with an average for the human samples of 30.4 ng/mL (The normal reference range for serum cortisone is 1.3-23 ng/mL[6].). Six normal human saliva samples were tested in the assay. Values ranged from 5.6 to 16.0 ng/mL with an average of 11.2 ng/mL. Eight normal human urine samples were also tested. The samples read from 49.4 to 268.3 ng/mL.

Dried fecal samples were processed as described on page 7 and run in the assay. Samples kindly donated by Dr. J. Williams at the Indianapolis Zoo, which included Amur Tiger, Giraffe, Kudu, Lion, Reeves Muntjac, White Handed Gibbon, White Rhino, and Zebra, were tested and cortisone values obtained ranged from 7.5 to 651 pg/mg dried fecal material.

Palme and Möestl and colleagues have shown that radiolabeled administered glucocorticoids are excreted in differing amounts in urine and feces[7] across species, with fecal excretion ranging from 7% of administered glucocorticoid in the pig to 82% in the cat[8-10]. Palme has also shown that the peak of fecal concentrations occur at 12 hours for sheep, but take 48 hours to peak in pigs. It is therefore necessary to evaluate the timing and relative fecal or urine excretion of glucocorticoids for each species.

9. Tietz, NW, In “Textbook of Clinical Chemistry”, WB Saunders, 1986.
10. Möstl, E., et al, Vet. Res. Commun. “Measurement of Cortisol Metabolites in Faeces or Ruminants.“ 2002, 26:127-139.
11. Palme, R., et al, Animal Reprod. Sci., “Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock.” 1996, 43:43-63.
12. Teskey-Gerstl, A., et al, J. Comp. Physiol. B, “Excretion of corticosteroids in urine and faeces of hares (Lepus europaeus).” 2000, 170: 163-168.
13. Schatz, S. and Palme, R., Vet. Res. Commun., Measurement of Faecal Cortisol Metabolites in Cats and Dogs: A Non-Invasive Method for Evaluating Adrenocortical Function.”, 2001, 25:271-287.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Cortisone	100%
5α-Dihydrocortisone	31.7%
Prednisone	9.0%
5β-Dihydrocortisone	4.4%
11-Dehydrocorticosterone	1.0%
Cortisol	< 0.1%
Progesterone	< 0.1%
Corticosterone	< 0.1%
Estradiol	< 0.1%
Dexamethasone	< 0.04%

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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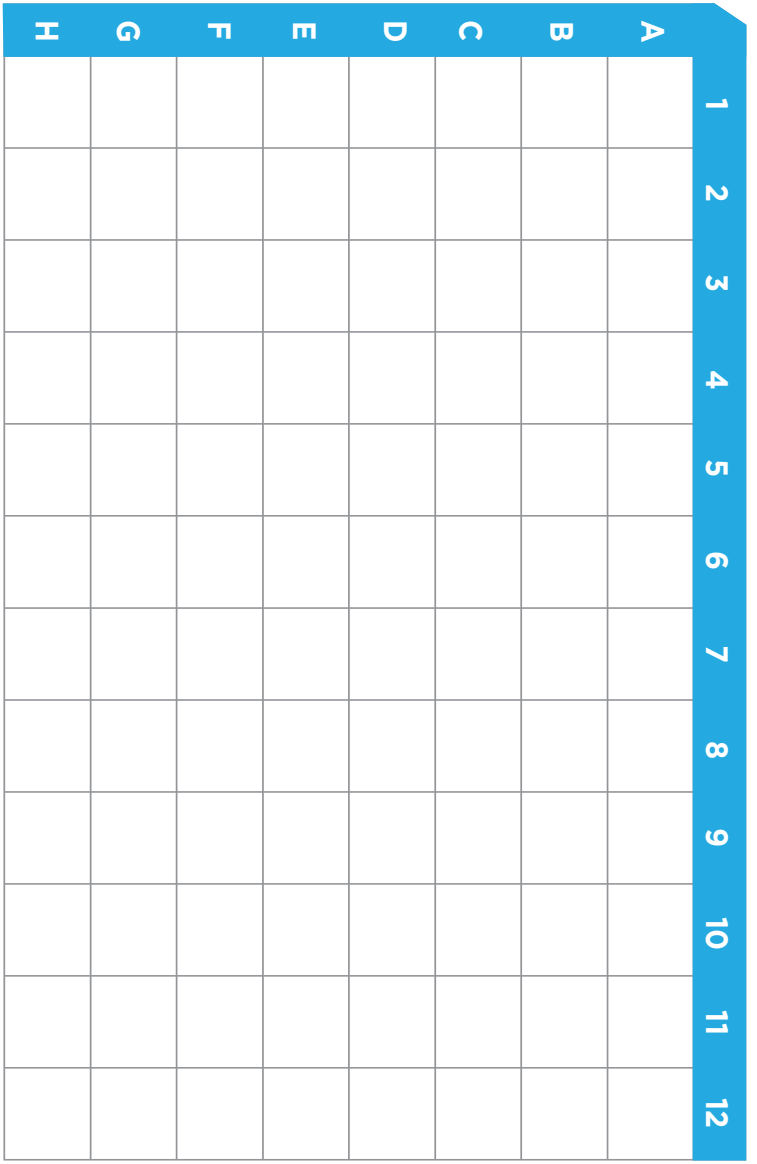
OFFICIAL SUPPLIER TO ISWE

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



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