ARBOR ASSAYS™

DetectX® Cortisol

ELISA Kit

1 Plate Kit – Catalog No. K003-H1
5 Plate Kit – Catalog No. K003-H5

Species Independent

Sample Types Tested:
Serum, EDTA and Heparin Plasma, Urine, Saliva,
Dried Fecal Extracts, and Tissue Culture Media

Calibrated to NIST Standard Reference Material Lot No. 921

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

		K003-H1	K003-H5	Description
Goat anti-Mouse Clear Coated 96-well Plate	Quantity	1	5	Strip well plates coated with goat anti-mouse IgG
	Catalog No.	X012-1EA	X012-1EA	
Cortisol Standard	Volume	125 µL	625 µL	Cortisol at 32,000 pg/mL in stabilizing solution
	Catalog No.	C040-125UL	C040-625UL	
DetectX® Cortisol Antibody	Volume	3 mL	13 mL	Mouse monoclonal antibody specific for Cortisol
	Catalog No.	C041-3ML	C041-13ML	
DetectX® Cortisol Conjugate	Volume	3 mL	13 mL	Cortisol-peroxidase conjugate in stabilizing solution
	Catalog No.	C039-3ML	C039-13ML	
Assay Buffer Concentrate	Volume	28 mL	55 mL	5X concentrate that must be diluted
	Catalog No.	X053-28ML	X053-55ML	
Dissociation Reagent	Volume	1 mL	5 mL	ONLY to be used with Serum and Plasma samples
	Catalog No.	X017-1ML	X017-5ML	
Wash Buffer Concentrate	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 peroxidase substrate
	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	

All components of this kit should 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. Repeater pipettes 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 (4PL) fitting. Contact your plate reader manufacturer for details.
- Optional: automated plate washer. Refer to Plate Washing Instructions for more details.
    - https://bit.ly/3tBT7N4

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/K003-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 peroxidase.
- **Take appropriate precautions when handling the Stop Solution, which is a caustic acid.**

BACKGROUND

Cortisol is the primary glucocorticoid produced and secreted by the adrenal cortex. Production of cortisol follows an ACTH-dependent circadian rhythm, with a peak level in the morning and decreasing levels throughout the day. Most of this circulating cortisol is bound to proteins such as corticosteroid-binding globulin and serum albumin.[1] Only free cortisol is available to interact with most receptors and modulate physiological processes.

Often referred to as the "stress hormone," cortisol affects blood pressure, blood sugar levels, and other actions of stress adaptation. Cortisol is an anti-inflammatory hormone playing roles in hypersensitivity, immunosuppression, and disease resistance.[2,3] Cortisol also promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization.[4] Abnormal cortisol levels correlate with depression, Cushing's Syndrome, Addison's Disease, diabetes and an increased risk of dementia and Alzheimer's disease.[5,6,7,8]

ASSAY PRINCIPLE

The Cortisol ELISA Kit quantitatively measures cortisol in serum, plasma, urine, saliva, dried fecal extracts, and tissue culture media samples. Total cortisol is measured in extracted samples, serum, and plasma; free cortisol is measured in urine and saliva. The Cortisol ELISA Kit is a competitive ELISA with a run time of 1.5 hours. Please read the complete kit insert for more information before performing this assay.

A cortisol stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Pipette the standards or diluted samples into a clear microtiter plate coated with goat anti-mouse IgG antibody. Add the cortisol peroxidase conjugate and the cortisol monoclonal mouse antibody. The immunological reaction occurs between the anti-cortisol monoclonal antibody, the cortisol antigen in the sample or standard, and the cortisol-peroxidase conjugate. Incubate the mixture for 1 hour, shaking at room temperature. Higher cortisol sample concentrations result in lower bound cortisol-peroxidase conjugate in the well, causing a decrease in signal and vice versa.

After the 1-hour incubation, wash away the excess cortisol-peroxidase and add the TMB substrate. The TMB substrate reacts with the bound cortisol-peroxidase conjugate generating a signal detected by a plate reader at 450nm. The concentration of cortisol in the sample is calculated, after making suitable correction for the dilution of the sample, 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 Concentrate 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 Concentrate 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

Collect samples at the same time of day, as cortisol levels vary throughout the day. For samples containing particulates, centrifuge prior to use. Upon collection, all samples should be frozen rapidly and stored at -80ºC until testing.

Sample Type	Procedure

Serum and Plasma	• Serum and Plasma samples must be treated with Dissociation Reagent.
	  ○ Handle samples carefully to avoid hemolysis.
	  ○ Allow Dissociation Reagent to warm to room temperature before use.
	• In a microcentrifuge tube:
	  ○ Add 5 µL Dissociation Reagent
	  ○ Add 5 µL Serum or Plasma
	  ○ Vortex gently to mix
	  ○ Incubate at room temperature for 5 minutes
	  ○ Add 490 µL 1X Assay Buffer to yield a 1:100 dilution
	  ▪ A minimum sample 1:100 dilution* is necessary to eliminate matrix interference.

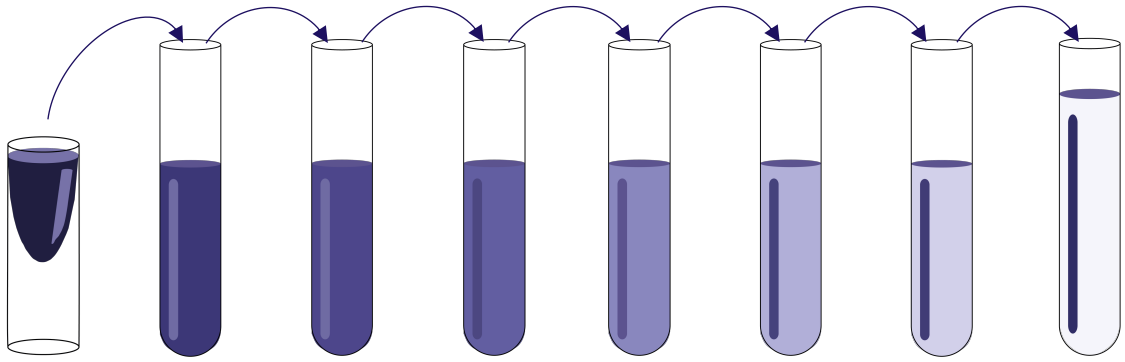
Dried Feces	• A detailed Extraction Protocol available on our website at: www.arborassays.com/resources/#protocols. • The ethanol concentration in the final sample added to the well should be <5%.
Urine	• Add 1 volume of urine to 7 volumes of 1X Assay Buffer to create an 8-fold dilution* • Use our Urinary Creatinine Detection kits (K002-H) to measure urine creatinine for normalization of cortisol 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. • Alternatively, prepare a minimum 2-fold dilution* of sample by adding 100 µL TCM to 100 µL 1X Assay Buffer.
Saliva	• Add 1 volume of saliva to 3 volumes of 1X Assay Buffer to create a 4-fold dilution*. • Saliva collection and handling instructions can be found on the Resource page at www.arborassays.com/resources/#protocols.

*Samples may require further dilution with 1X Assay Buffer to fall within the standard curve range.

⚠ **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 250 µL 1X Assay Buffer to Standard 2 – 7 tubes.
4. Add 50 µL of the Cortisol Standard stock solution to Standard 1 tube. Vortex thoroughly.
   ⚠ **The Cortisol Standard stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.**
5. Transfer 250 µL of Standard 1 into Standard 2 tube. Vortex thoroughly.
6. Transfer 250 µL of the mixed solution from Standard 2 into Standard 3 tube. 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	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	50	250	250	250	250	250	250
Final Concentration (pg/mL)	3,200	1,600	800	400	200	100	50

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

ASSAY PROTOCOL

Before You Begin:

- **Room Temperature for this assay is defined as 22ºC – 24ºC.**
- **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 for a 96-well Corning CoStar 2592 plate. See ArborAssays.com for plate dimension data.
- Determine the number of well strips to be used and return unused well strips to 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 of Samples or Standards into duplicate wells.
2. Add 75 µL of 1X Assay Buffer into duplicate non-specific binding (NSB) wells.
3. Add 50 µL of 1X Assay Buffer into duplicate Zero Standard (maximum binding or B0) wells.
4. Add 25 µL of DetectX® Cortisol Conjugate to each well.
5. Add 25 µL of DetectX® Cortisol Antibody to each well, **except the NSB wells.**
6. Cover the plate with the plate sealer and shake at room temperature at 700-900 rpm for **1 hour**.
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.
    - 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.
    - 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 this online tool: https://myassays.com/assay.aspx?id=1234

1. Use four-parameter logistic curve (4PL) software to calculate the cortisol concentration for each sample. Gather all raw data OD readings from each Sample and Standard, including the Zero (B0) Standard and NSB.
2. 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	0.040	0.060	0.050
B0	0.939	0.945	0.942
Sample 1	0.356	0.366	0.361

3. 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	0.942	0.050	0.892
Sample 1	0.361	0.050	0.311

4. 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	0.311	0.892	34.9

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

   Use the sample %B/B0 readings and the 4PL fit to calculate cortisol concentrations in diluted samples. If diluted sample 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 Cortisol Concentration (pg/mL)
Sample 1	0.311	34.9	1,583

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

EXAMPLE:

Sample	Sample Cortisol Concentration (pg/mL)	Sample Dilution Factor	Original Sample Cortisol Concentration (pg/mL)
Sample 1	1,583	100	158,300

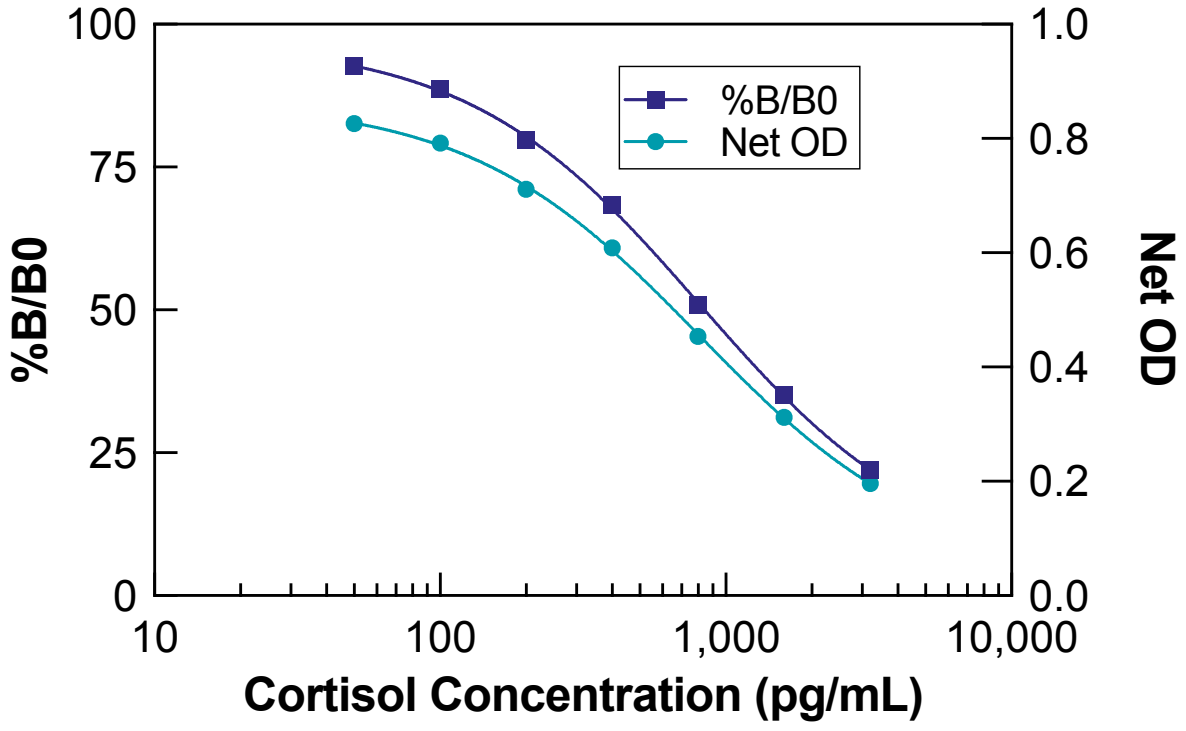
TYPICAL DATA

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

Sample	Mean OD	Net OD	%B/B0	Cortisol Concentration (pg/mL)
NSB	0.050	-	-	-
Standard 1	0.246	0.196	22.0	3,200
Standard 2	0.362	0.312	35.0	1,600
Standard 3	0.504	0.454	50.9	800
Standard 4	0.659	0.609	68.3	400
Standard 5	0.761	0.711	79.7	200
Standard 6	0.842	0.792	88.7	100
Standard 7	0.876	0.826	92.6	50
B0	0.942	0.892	100	0
Sample 1	0.361	0.311	34.9	1,583
Sample 2	0.590	0.540	60.5	543

Conversion Factor: 100 pg/mL of Cortisol is equivalent to 275.9 pM.

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 36 pg/mL.

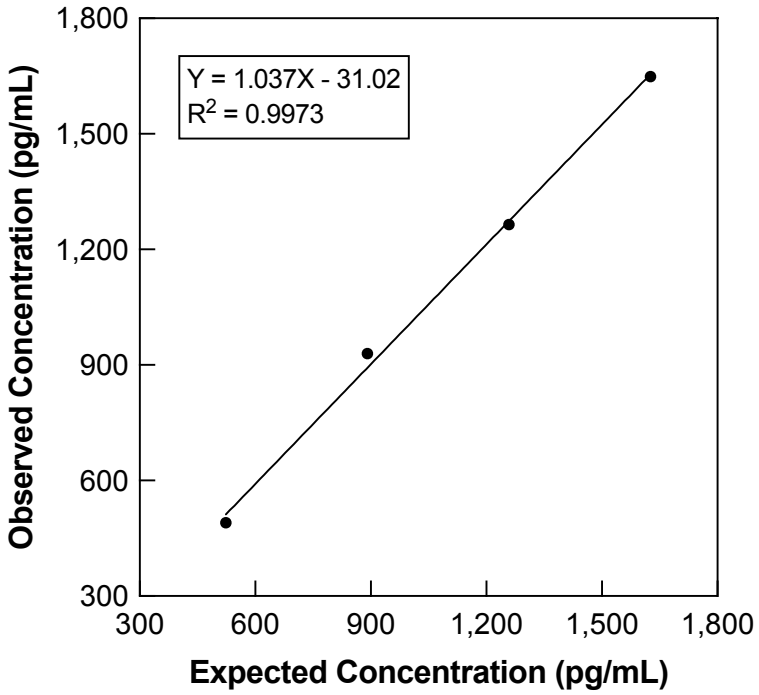
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the B0 and a low concentration human sample.

The Limit of Detection was determined as 57 pg/mL.

Linearity

Linearity was determined in human urine by diluting two samples with known cortisol concentration with 1X Assay Buffer. One sample had a cortisol concentration of 156.5 pg/mL (Low Sample); a second sample had a cortisol concentration of 1,993.5 pg/mL (High Sample). 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 Sample	High Sample	Expected Concentration (pg/mL)	Observed Concentration (pg/mL)	% Recovery
80%	20%	523.9	490.5	101.4
60%	40%	891.3	929.9	100.4
40%	60%	1,258.7	1,264.2	104.3
20%	80%	1,626.1	1,648.5	93.6
			Mean Recovery	**99.9%**



Intra Assay and Inter Assay Precision

For intra assay precision, one human plasma sample and one human serum sample were diluted in 1X Assay Buffer and run in replicates of 20 in an assay. For inter assay precision, a second human plasma sample and a second human serum sample were diluted in 1X Assay Buffer and run in duplicates in 24 assays run over multiple days by five operators. %CV represents the variation in concentration (not optical density) as determined using a standard curve.

	Intra Assay Precision		Inter Assay Precision	
Sample	Cortisol Concentration (pg/mL)	% CV	Cortisol Concentration (pg/mL)	% CV
Plasma	1,736	6.2	1,584	11.0
Serum	619	10.1	541	12.0

SAMPLE VALUES

3 mammalian serum samples were treated with Dissociation Reagent, diluted in 1X Assay Buffer, and tested in the assay. 3 mammalian plasma samples were treated with Dissociation Reagent, diluted in 1X Assay Buffer and tested in the assay. 3 mammalian urine samples were diluted with 1X Assay Buffer and tested in the assay. 2 dried mammalian fecal samples were extracted, diluted with 1X Assay Buffer, and tested in the assay. The adjusted average concentration and ranges are shown below.

Sample Type	Average Adjusted Concentration (ng/mL)	Adjusted Concentration Range (ng/mL)
Serum	101.4	91.3 – 120.6
Plasma	197.8	83.3 – 403.9
Urine	195.4	90.3 – 260.3
Dried Feces	32.5	26.0 – 39.1

INTERFERENCE

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

Interferent	Effect at High Cortisol Concentration		Effect at Low Cortisol Concentration	
	% Added	Effect	% Added	Effect
DMSO	5%	5.3% increase	5%	3.2% decrease
Ethanol	2.5%	4.3% decrease	2.5%	4.6% decrease
Methanol	1.25%	10.5% decrease	1.25%	2.8% decrease
DMF	5%	6.2% decrease	5%	9.0% increase
Hemoglobin	40 mg/dL	0.2% decrease	40 mg/dL	7.2% decrease
Bilirubin	5 mg/dL	1.6% decrease	5 mg/dL	4.5% decrease

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Cortisol	100%
Dexamethasone	19.5%
Prednisolone (1-Dehydrocortisol)	10.2%
Corticosterone	1.4%
Progesterone	<0.1%
Estradiol	<0.1%
Cortisol 21-Glucuronide	<0.1%
1α-hydroxycorticosterone	<0.1%
Testosterone	<0.1%
Cortisone	0.4%

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 Background	• Ensure assay plate has been properly washed with the number of washes indicated in the protocol. • Reagent contamination during assay setup.
Low Signal	• Confirm tools, equipment, reagents, and containers used do not contain any trace of sodium azide. • Altering the shaking speeds or excluding shaking during incubation steps. • Verify the plate reader wavelength is 450 nm.

CITATIONS

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8. Ouanes, Sami., Popp, Julius. (2019). High cortisol and the risk of dementia and Alzheimer’s disease: A review of the literature. Frontiers in Aging Neuroscience, 2019; 11:43
9. Samples kindly donated by Dr. J. Williams at the Indianapolis Zoo.

RELATED PRODUCTS

Kits	Catalog No.
Corticosterone ELISA Kits	K014-H1/H5
Cortisone Chemiluminescent Immunoassay Kits	K017-C1/C5
Cortisone ELISA Kits	K017-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

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.

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CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us.

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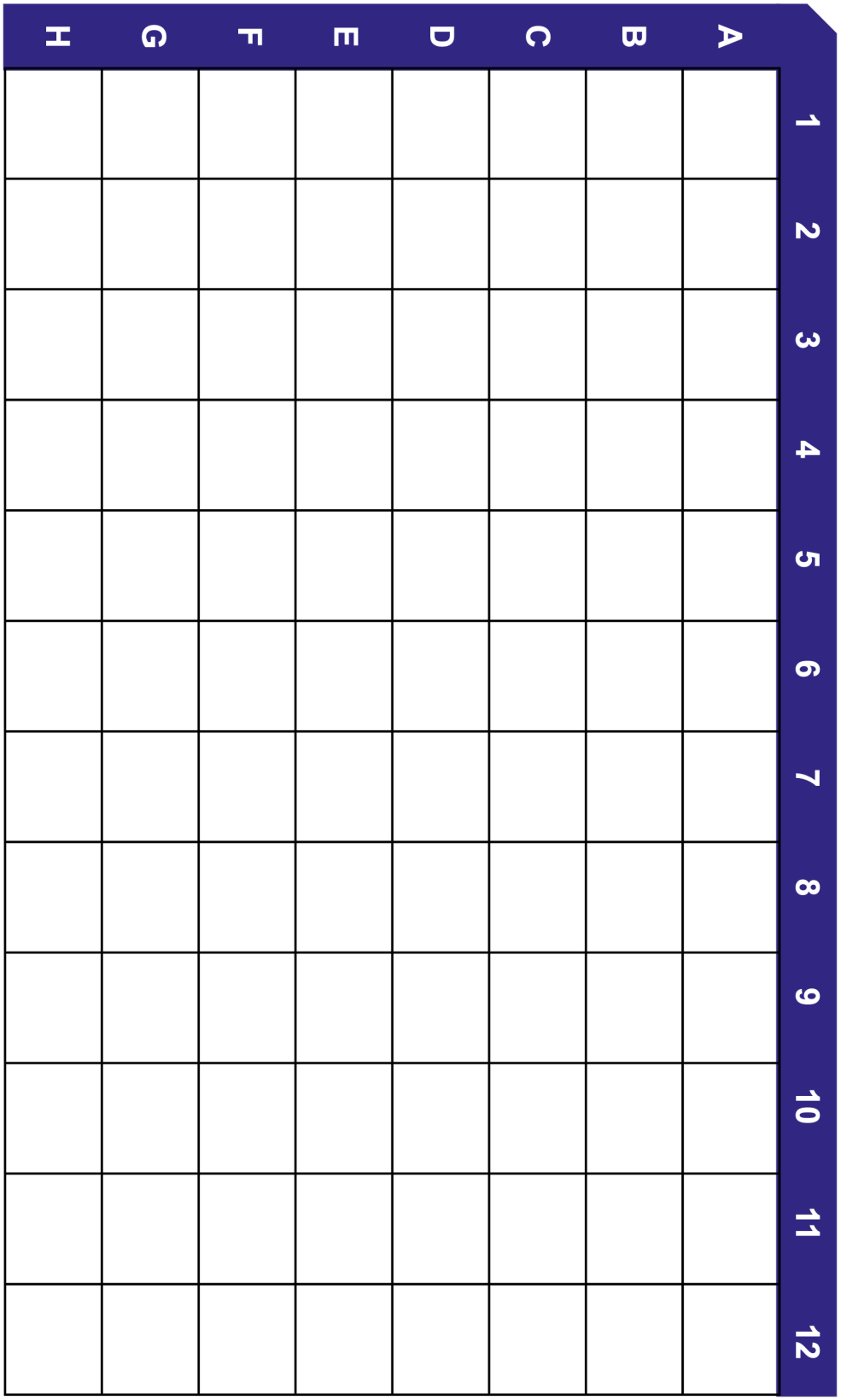
OFFICIAL SUPPLIER TO ISWE

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.

PLATE LAYOUT



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