DetectX® Acetylcholinesterase (AChE)

Fluorescent Activity Kit

2 Plate Kit – Catalog No. K015-F1

Species Independent

Sample Types Tested:

Serum, Plasma, and Erythrocyte Membranes

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

TABLE OF CONTENTS

SUPPLIED COMPONENTS & STORAGE

		K015-F1	Description
Black 96-well Plate	Quantity	2	Non-treated 96-well plates
	Catalog No.	X001-2EA	
Acetylcholinesterase Standard	Volume	225 µL	Recombinant Acetylcholinesterase (AChE) at 1,000 mU/mL in stabilizing solution
	Catalog No.	C046-225UL	
ThioStar® Detection Reagent	Quantity	2 vials	Store in sealed pouch with desiccant. Must be reconstituted with dry DMSO.
	Catalog No.	C048-1EA	
Dry DMSO (Anhydrous)	Volume	14 mL	Dimethyl sulfoxide, dried over molecular sieves. **May be stored at room temperature*.**
	Catalog No.	X022-14ML	
Assay Buffer Concentrate 10x	Volume	28 mL	10X concentrate that must be diluted
	Catalog No.	X064-28ML	
AChE Substrate	Quantity	2 vials	Acetylthiocholine iodide. Must be reconstituted with dry DMSO.
	Catalog No.	C047-1EA	

*When stored at 4ºC, DMSO will freeze. It can be stored tightly capped at room temperature.

This kit should be stored at 4ºC up to the expiration date on the kit label**.**

OTHER MATERIALS REQUIRED

- Distilled or deionized water
- Adjustable pipettes with disposable tips capable of dispensing 50 µL, 100 µL, and 200 µL. Repeater pipettes or multichannel pipettes with corresponding tips are also recommended.
- High-quality polypropylene test tubes for Standard and Sample preparation. **Do not use glass tubes**.
- Amber vial, sufficient to contain Reaction Mix
- Microcentrifuge
- 96-well Plate Shaker, sufficient to shake between 700 – 900 rpm.
- A fluorescence plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm. Contact your plate reader manufacturer for correct filter sets.
- Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and performing a linear regression analysis. Contact your plate reader manufacturer for details.

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/K015-F1_MSDS.pdf
- Ensure all buffers used for samples are azide free. ThioStar® will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™, and Kathon™ will react with the substrate.

BACKGROUND

Acetylcholinesterase (AChE) is an enzyme responsible for the breakdown of acetylcholine, a key neurotransmitter that regulates signaling in cholinergic neurons within the central and peripheral nervous systems[1]. Through hydrolysis, AChE terminates cholinergic transmission at synaptic and neuromuscular junctions, preventing overstimulation of cholinergic receptors and allowing for precise control of neural communication. The activity of AChE is essential for maintaining normal neural function[1], development[2], and circadian rhythm regulation[3].

Research into AChE activity provides valuable insights into neurological health and disease progression. For example, the enzyme's function is notably impacted in Alzheimer's disease, where impaired cholinergic transmission is a hallmark[4]. Quantitative measurement of AChE activity in blood, cerebrospinal fluid, and tissue samples is instrumental in studying neurodegenerative conditions and understanding how AChE modulation can alleviate symptoms[5]. AChE inhibitors, which slow acetylcholine breakdown and enhance cholinergic signaling, are widely used in Alzheimer's disease treatment protocols.

Beyond clinical applications, measuring AChE activity is significant in agricultural and environmental toxicology due to its susceptibility to inhibition by certain pesticides. Organophosphates and carbamates, commonly used insecticides, block AChE function, leading to excessive accumulation of acetylcholine, with toxic effects on nervous systems across species[6]. Researchers measure AChE activity to assess exposure levels, monitor toxic effects, and understand ecological impacts.

The DetectX® AChE Fluorescent Activity kit enables precise, quantitative measurement of enzyme activity in a variety of sample types. This allows researchers to examine AChE's role across species and in diverse physiological and pathological contexts, supporting advancements in neuroscience, toxicology, and therapeutic development.

ASSAY PRINCIPLE

The DetectX® Acetylcholinesterase (AChE) Fluorescent Activity Kit is designed to quantitatively measure AChE activity in a variety of samples. Please read the complete kit insert before performing this assay.

The kit utilizes a proprietary non-fluorescent molecule, ThioStar®, that covalently binds to the thiol product of the reaction between the AChE Substrate and AChE in the Standards or Samples, yielding a fluorescent product read at 510 nm in a fluorescent plate reader with excitation at 390 nm.

A human AChE standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are diluted in 1X Assay Buffer and added to the wells of the plate. The reaction is initiated with the addition of the Reaction Mix containing AChE Substrate and ThioStar® Reagent. The reaction is incubated with shaking for 20 minutes and the fluorescent signal is read.

The kit is suitable for measuring AchE activity in diluted serum, plasma, and Erythrocyte Membranes.

REAGENT PREPARATION

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

Reagent	Preparation	Stability
1X Assay Buffer	Warm 10X Assay Buffer Concentrate to room temperature and mix thoroughly by inversion. Mix 1 volume 10X Assay Buffer Concentrate with 9 volumes deionized water.	1X Assay Buffer is stable for 3 months at 4°C.
10X ThioStar® Detection Reagent	Warm ThioStar® Detection Reagent to room temperature and pulse spin in a microcentrifuge. Add 700 µL of the provided DMSO and nutate for 5 minutes to mix thoroughly.	Reconstituted ThioStar® is stable for 2 months at 4°C.
10X Acetylcholinesterase Substrate	Warm the Acetylcholinesterase Substrate to room temperature and pulse spin in a microcentrifuge. Add 700 µL of the provided DMSO and nutate for 5 minutes to mix thoroughly.	Reconstituted substrate is stable for 2 months at 4°C.

SAMPLE PREPARATION

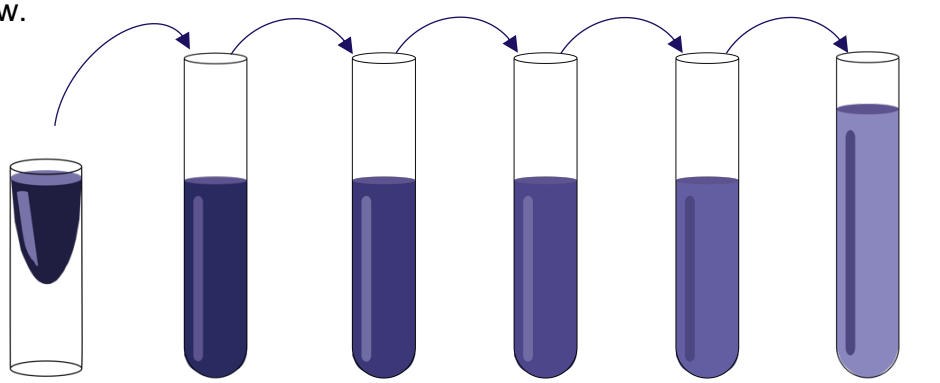
For samples containing particulates, centrifuge prior to use. Upon collection, all samples should be frozen rapidly and stored at -80˚C until testing.

Sample Type	Procedure
Serum and Plasma	• Prepare a ≥ 300-fold dilution of sample by diluting each sample in 1X Assay Buffer. • Samples may require further dilution with 1X Assay Buffer to fall within the standard curve range.
Erythrocyte Membranes	• Collect blood in heparin or EDTA tubes. Centrifuge the sample at 700 x g for 15 minutes at 4ºC. Aspirate the plasma and white cell layer and use it as described above or discard. • Gently wash the erythrocytes twice with three volumes of isotonic saline (0.9%). Centrifuge the sample at 700 x g for 10 minutes and discard the saline after each wash. • Lyse the erythrocytes by adding four volumes of cold, deionized water. Vortex cells and incubate for 10 minutes at 4ºC. • Centrifuge at 14,000 rpm for 10 minutes at 4ºC. Remove and discard the supernatant. • Wash the membrane pellet until it is faintly pink. To wash, add three volumes of isotonic saline to the tube, vortex, and centrifuge at 14,000 rpm for 15 minutes. Remove and discard the saline between washes. The smaller dark red pellet is non-lysed RBCs and should be avoided. • Solubilize the white membrane pellet with Triton X-100 by carefully pipetting up and down and transfer to a clean tube. • Prepare a minimum 100-fold dilution of Erythrocyte Membranes by diluting the sample in 1X Assay Buffer before adding to the assay • 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 5.
2. Pulse spin the Acetylcholinesterase Standard in a microcentrifuge.
3. Add 450 µL 1X Assay Buffer to Standard 1 tube.
4. Add 250 µL 1X Assay Buffer to Standard 2 – 5 tubes.
5. Add 50 µL of the AChE Standard stock solution to Standard 1 tube. Vortex thoroughly.
6. Transfer 250 µL of Standard 1 into Standard 2 tube to make a 2-fold dilution. Vortex thoroughly.
7. Transfer 250 µL of the mixed solution from Standard 2 into Standard 3 tube to make a 2-fold dilution. Vortex thoroughly.
8. Continue serially diluting into the remaining tubes. This process and the final activities are summarized in the table below.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
1X Assay Buffer (µL)	450	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4
Volume of Addition (µL)	50	250	250	250	250
Final Activity (mU/mL)	100	50	25	12.5	6.25

⚠ **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.
- Set plate parameters on the plate reader for a 96-well Corning CoStar 3915 plate. See ArborAssays.com for plate dimension data.

1. Add 100 µL Samples or Standards into duplicate wells. Add 100 µL 1X Assay Buffer into duplicate wells as the Zero Standard.
2. Prepare the Reaction Mix in an amber vial and protected from light as described in the table below. Vortex briefly to mix and proceed immediately to Step 3.

	Half Plate	Full Plate
10X ThioStar Detection Reagent	300 µL	550 µL
10X Acetylcholinesterase Substrate	300 µL	550 µL
DMSO	2.4 mL	4.4 mL

3. Add 50 µL of the prepared Reaction Mix to each well.
   ⚠ Dispense reagents in a smooth motion and monitor the tip closely to ensure that all reagents are transferred.
4. Shake the plate (700 – 900 rpm) at room temperature for 20 minutes.
5. After the 20-minute incubation, read the plate immediately using a fluorescent emission at 510 nm with excitation at 370 – 410 nm.

CALCULATION OF RESULTS

Follow the instructions below or use the online tool: https://www.myassays.com/assay.aspx?id=901

1. Use linear regression software to calculate the AChE activity for each Sample. Gather all raw data FLU readings from each Sample and Standard, including the Zero Standard.
2. Average the duplicate FLU readings for each Sample, Standard, and Zero Standard (Mean FLU).

EXAMPLE:

Sample	Replicate 1 FLU	Replicate 2 FLU	Mean FLU
Zero Standard	2,369	2,389	2,379
Standard 1	51,495	51,503	51,499
Sample 1	23,732	23,772	23,752

3. Subtract the Zero Standard Mean FLU from the Mean FLU for each Sample and Standard (Net FLU).

EXAMPLE:

Sample	Mean FLU	Zero Standard Mean FLU	Net FLU
Standard 1	51,499	2,379	49,120
Sample 1	23,752	2,379	21,373

4. Plot the standard curve with Net FLU for the Standards on the y-axis and AChE activity (mU/mL) on the x-axis. Perform a linear regression.

   Use the slope and Y-intercept of the regression line, together with the Net FLU to calculate the AChE activity in the diluted Samples using the equation below. If diluted AChE activities are outside of the range of the Standards, the Sample should be prepared again at a more appropriate dilution.

$$\text{Sample AChE Activity (mU/mL)} = \frac{\text{(Net FLU)} - \text{(Y-intercept)}}{\text{Slope}}$$

EXAMPLE:

Sample	Net FLU	Sample AChE Activity (mU/mL)
Sample 1	21,373	43.5

5. If the original sample was diluted, multiply the AChE activity by the Sample Dilution Factor to determine the activity of AChE in the original sample.

EXAMPLE:

Sample	Sample AChE Activity (mU/mL)	Sample Dilution Factor	Original Sample AChE Activity (mU/mL)
Sample 1	43.5	300x dilution	13,050

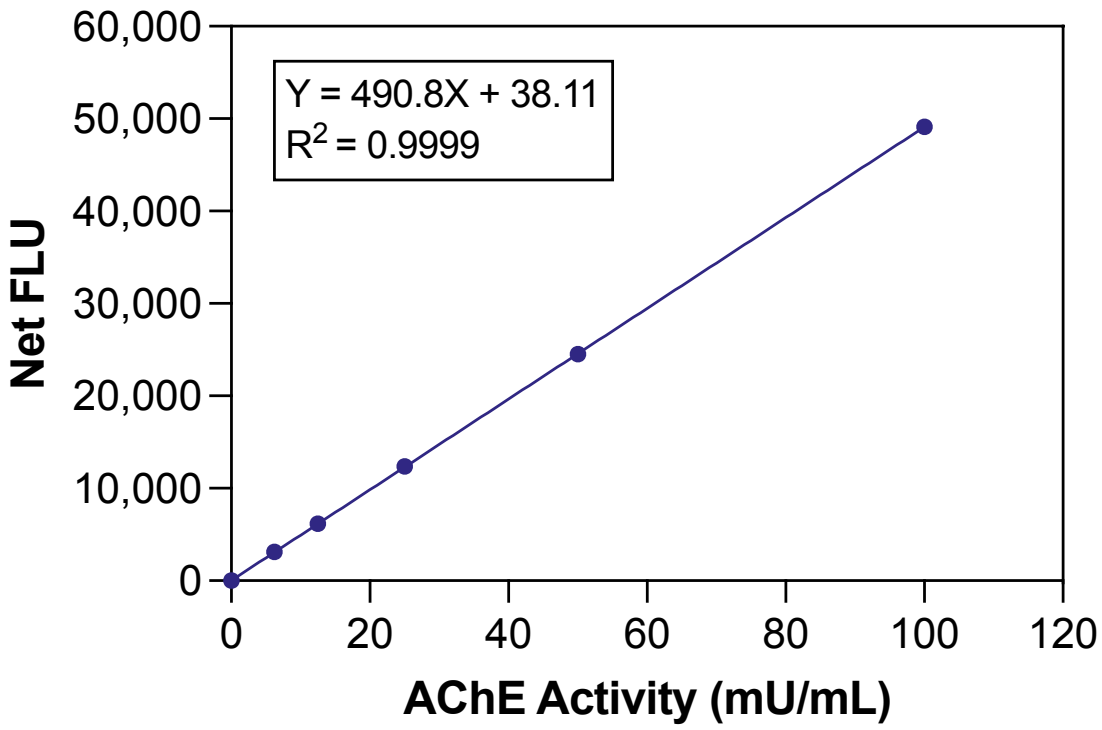
TYPICAL DATA

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

Sample	Mean FLU	Net FLU	AChE Activity (mU/mL)
Standard 1	51,499	49,120	100
Standard 2	29,926	24,548	50
Standard 3	14,753	12,375	25
Standard 4	8,543	6,165	12.5
Standard 5	5,496	3,118	6.23
Zero	2,379	0	0
Sample 1	23,752	21,374	43.4
Sample 2	11,915	9,536	19.4

AChE Unit Definition
One unit of AChE is defined as the amount of enzyme needed to hydrolyze 1.0 micromole of acetylthiocholine iodide per minute at 25ºC.[7]

Typical Standard Curve



VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and Standard 5. The detection limit was determined at two standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.063 mU/mL.

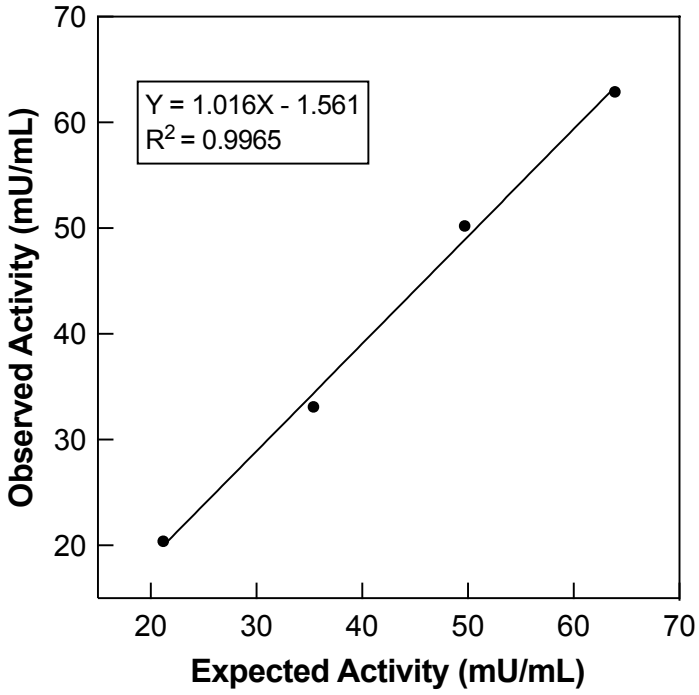
The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the Zero Standard and a low activity sample.

Limit of Detection was determined as 0.109 mU/mL.

Linearity

Linearity was determined in human serum by diluting two samples with known AChE activity with 1X Assay Buffer. One sample had an AChE activity of 6.9 mU/mL (Low Sample), and a second had an AChE activity of 78.2 mU/mL (High Sample). The two samples were mixed in the ratios given below and the measured activities were compared to the expected values for each given ratio.

Low Sample	High Sample	Expected Activity (mU/mL)	Observed Activity (mU/mL)	% Recovery
80%	20%	21.2	20.4	96.4
60%	40%	35.4	33.1	93.5
40%	60%	49.7	50.2	101.0
20%	80%	63.9	62.9	98.4
			Mean Recovery	**97.3**



Intra Assay and Inter Assay Precision

For intra assay precision, two serum and one plasma sample were diluted in 1X Assay Buffer, and 22 replicates were run in one assay. For inter assay precision, two serum and one plasma sample were diluted in 1X Assay Buffer and duplicates of each sample were run in 21 assays over multiple days by 5 operators. The %CV represents the variation in activity (not fluorescence level) as determined using a standard curve.

	Intra Assay Precision		Inter Assay Precision	
Sample	AChE Activity (mU/mL)	% CV	AChE Activity (mU/mL)	% CV
1	45.3	2.6	43.4	6.9
2	19.5	3.8	19.4	6.1
3	5.7	4.4	6.5	12.3

SAMPLE VALUES

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

Sample Type	Recommended Minimum Dilution	Adjusted Average Activity (mU/mL)	Adjusted Activity Range (mU/mL)
Serum	1:300	10,789	6,608 – 16,085
Plasma	1:300	9,039	5,341 – 11,502

INTERFERENCE

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

Interferent	Effect
DMSO (5.0%)	12.6% decrease
Ethanol (5.0%)	6.4% increase
Ethanol (10.0%)	43.0% decrease
DMF (5.0%)	19.7% decrease
Methanol (10%)	6.3% decrease

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 plate is shaking at 700 – 900 rpm during the 20-minute incubation step. • Prepare reagents in high quality polypropylene tubes. Do not use glass tubes. • Prerinse pipet tips with desired reagent prior to aspirating the required volume. • Deliver volume with care to prevent splashing into adjacent wells. • Read plate immediately at the 20-minute timepoint. Since this assay does not include a stop solution, the reaction will continue even after 20 minutes. • Use the Reaction Mix within 30 minutes of preparation. • Always make fresh Reaction Mix; never store Reaction Mix for future use.
Low Signal	• Confirm tools, equipment, reagents, and containers used do not contain any trace of sodium azide. • Verify the plate reader is set to fluorescent emission at 510 nm with excitation at 370-410 nm. • Confirm reagents are at room temperature prior to use.
Measuring AChE vs BChE	• The cross reactivity of Butyrylcholinesterase (BChE) in this assay is 150%. As such, measuring AChE in a sample where both AChE and BChE are present could result in artificially high activity values of AChE. • To measure only AChE in a mixed sample: 1. Run the same sample in both the K015-F1 (AChE) and K016-F1 (BChE) kits from Arbor Assays. 2. Multiply the BChE value obtained from K016-F1 by the cross-reactivity factor (1.5) 3. Subtract the BChE value from step 2 from the total value obtained using the K015-F1 kit. *Example:* Measured Activity from K015-F1 (Step 1): 65 mU/mL Measured Activity from K016-F1 (Step 1): 10 mU/mL Cross reactivity (Step 2): 10 mU/mL * 1.5 = 15 mU/mL Calculated AChE (Step 3): 65 mU/ mL – 15 mU/mL = 50 mU/mL

CITATIONS

1. Trang A, Khandhar PB. (2023) Physiology, Acetylcholinesterase. *StatPearls* [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539735/
2. Bigbee JW, Sharma KV, Gupta JJ, Dupree JL. (1999) Morphogenic role for acetylcholinesterase in axonal outgrowth during neural development. Environ Health Perspect. 107 Suppl 1(Suppl 1):81-7.
3. Gao X, Zhang M, Lyu M, Lin S, Luo X, You W, Ke C. (2022). Role of Bmal1 in mediating the cholinergic system to regulate the behavioral rhythm of nocturnal marine molluscs. Comput Struct Biotechnol J. 23;20:2815-2830.
4. García-Ayllón, M. S., Small, D. H., Avila, J., & Sáez-Valero, J. (2011). Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with P-tau and β-amyloid. Frontiers in molecular neuroscience, 4, 22.
5. Walczak-Nowicka, Ł. J., & Herbet, M. (2021). Acetylcholinesterase inhibitors in the treatment of neurodegenerative diseases and the role of acetylcholinesterase in their pathogenesis. International journal of molecular sciences, 22(17), 9290.
6. Vale, A., & Lotti, M. (2015). Organophosphorus and carbamate insecticide poisoning. Handbook of clinical neurology, 131, 149-168.
7. Järvinen, Paivi P.; Fallarero, Adyary; Gupta, Shikhar; Mohan, Gobi C.; Hatakka, Annele I.; Vuorela, Pia M. (2010). Miniaturization and Validation of the Ellman's Reaction Based Acetylcholinesterase Inhibitory Assay into 384-Well Plate Format and Screening of a Chemical Library. *Combinatorial Chemistry & High Throughput Screening*, Volume 13, Number 3, 2010, pp. 278-284(7)

RELATED PRODUCTS

Kits	Catalog No.
Butyrylcholinesterase Fluorescent Activity Kit	K016-F1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Hemoglobin Colorimetric Detection Kit	K013-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Histone Demethylase Fluorescent Activity Kit	K010-F1
P450 Fluorescent Activity Kit	K011-F1

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

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

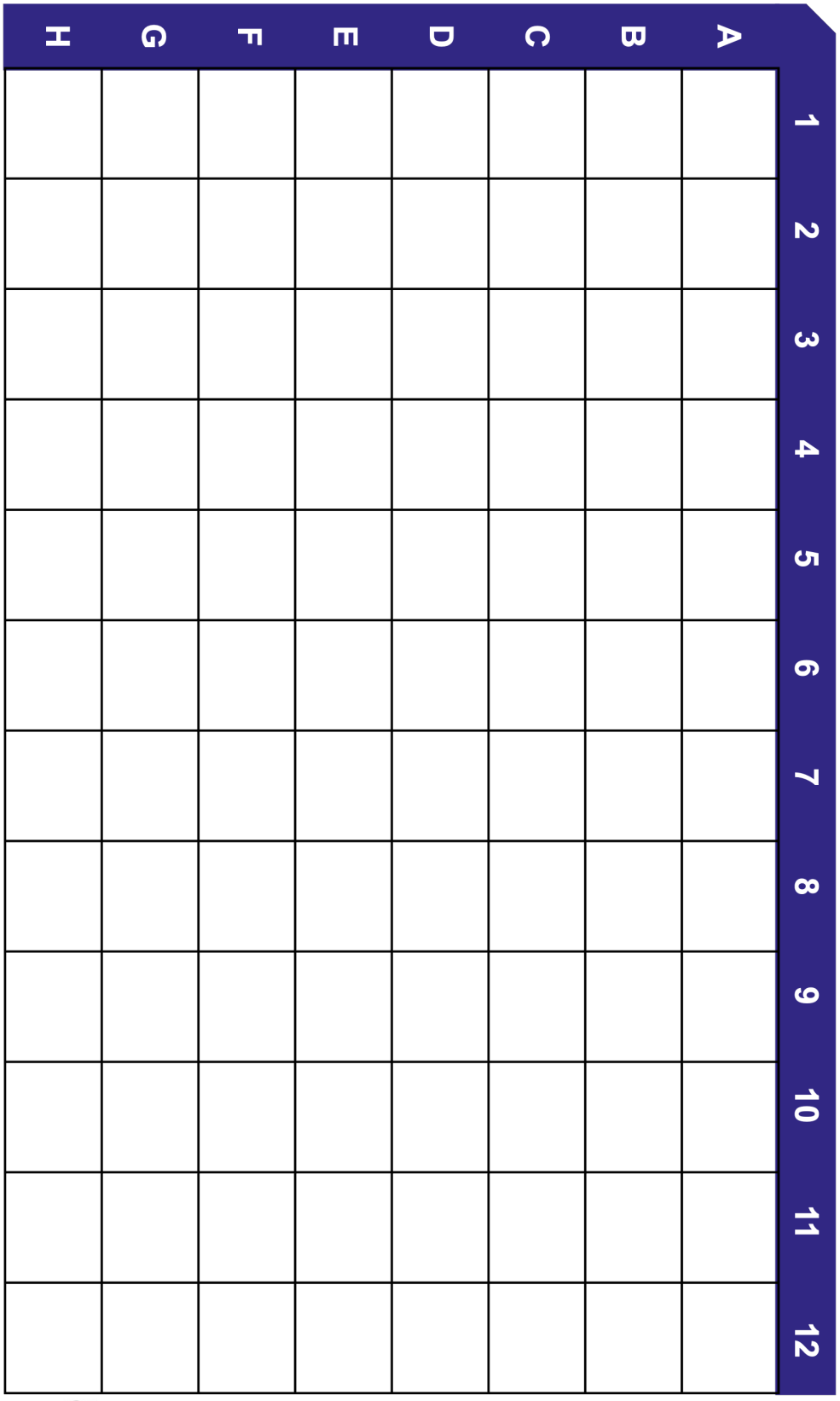
Orders@ArborAssays.com

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



FSC Printed on Forest Stewardship Council certified paper