ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Butyrylcholinesterase
Fluorescent Activity Kit

2 Plate Kit Catalog Number K016-F1

Species Independent

Sample Types Validated:

Serum and Plasma

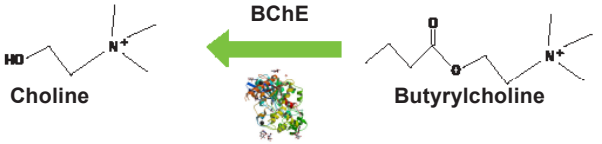
Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

BACKGROUND

Butyrylcholinesterase (BChE) belongs to the same structural class of proteins as acetylcholinesterase (AChE). The 440kDa tetrameric glycoprotein is predominantly found in blood, kidneys, intestine, liver, lung, heart and the central nervous system. Many species, such as human, horse and mice exhibit high BChE activity in plasma, whereas rats have higher acetylcholinesterase activity in plasma[1]. BChE preferentially acts on butyrylcholine, but also hydrolyzes acetylcholine.



BChE serves a few known functions within the body. As a detoxification enzyme, it hydrolyzes ester-containing drugs and scavenges cholinesterase inhibitors, such as succinylcholine, before they have a chance to reach synaptic targets. By doing this, the enzyme minimizes the neuromuscular effect these agents have. A deficiency of BChE can result in delayed metabolism of various drugs, such as cocaine, and treatment with doses of BChE can help in overcoming the physiological reaction to them[2]. As an activator enzyme, BChE converts administered prodrugs into functional therapeutics. Bambuterol is a prodrug with anti-asthmatic properties after being converted by BChE. BChE is the only enzyme in human serum that acts on heroin, and its end product, after crossing the blood-brain barrier, is hydrolyzed to morphine by enzymes in the brain.

Alzheimer's disease involves the degeneration of cholinergic neurons and loss of cholinergic transmission. The reduction in choline acetyltransferase leads to a decrease in acetylcholine and acetylcholinesterase activity, which appears to cause an increase in BChE activity[3]. Potent cholinesterase inhibitor therapeutics protect the limited acetylcholine levels, acting on both AChE and BChE. Selective BChE inhibitors prevent the formation of new beta-amyloid plaques, which are created by BChE cleaving amyloid precursor protein to beta-amyloid protein[4]. BChE-positive neurons project to the frontal cortex portion of the brain. BChE may have roles in attention, executive function, emotional memory and behaviour. As dementia advances, BChE activity has been shown to increase, while AChE activity decreases, leaving the potential for BChE activity to be used as a biomarker for progression[5] or target for future therapies.

1. Çokuğraş, A. N. (2003). Butyrylcholinesterase: Structure and physiological importance. *Turkish Journal of Biochemistry, 28*(2), 54–61.
2. Carmona, G. N., et al. (1999). Butyrylcholinesterase accelerates cocaine metabolism: In vitro and in vivo effects in nonhuman primates and humans. *Drug Metabolism and Disposition, 28*(3), 367–371.
3. Allam, A. R., et al. (2006). Alzheimer's disease and Type 2 Diabetes mellitus: The cholinesterase connection? *Lipids in Health and Disease, 5*(1), 28.
4. Greig, N. H., et al. (2005). Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer β-amyloid peptide in rodent. *Proceedings of the National Academy of Sciences, 102*(47), 17213–17218.
5. Lane, R. M., et al. (2006). Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *International Journal of Neuropsychopharmacology*, *9*(1), 101–124.

ASSAY PRINCIPLE

The DetectX® Butyrylcholinesterase Activity Kit is designed to quantitatively measure butyrylcholinesterase (BChE) activity in a variety of samples. Please read the complete kit insert before performing this assay. A human BChE standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a proprietary non-fluorescent molecule, ThioStar®, that covalently binds to the thiol product of the reaction between the BChE Substrate and BChE in the standards or samples, yielding a fluorescent product read at 510 nm in a fluorescent plate reader with excitation at 390 nm.

The kit is suitable for measuring BChE activity in appropriately diluted serum or plasma from a number of species. It will also measure BChE in extracted tissue samples, CSF and cell lysates. Because the readout of BChE activity is purely chemical, there are few interferants that will affect the readings obtained.

REACTION OVERVIEW

1. Sample or standard added to well.
2. The reaction is initiated with the addition of the Reaction Mix containing BChE Substrate and ThioStar® Reagent.
3. Incubate for 20 minutes and read fluorescent signal. Calculate BChE activity from standard curve.
4. Alternatively samples can be read kinetically. Follow steps 1 and 2 above.
5. Add Reaction Mix and read signal at 510 nm over time. Compare rates for samples and standards to determine sample BChE activity.

RELATED PRODUCTS

Kits	Catalog No.
Acetylcholinesterase Fluorescent Activity Kit	K015-F1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione S-Transferase Fluorescent Activity Kit	K008-F1
Histone Demethylase Fluorescent Activity Kit	K010-F1
P450 Fluorescent Activity Kit	K011-F1

SUPPLIED COMPONENTS

Black 96 Well Plates
See: www.ArborAssays.com/resources/#general-info for plate dimensions.

2 Plates Catalog Number X025-2EA

Butyrylcholinesterase Standard
Butyrylcholinesterase (BChE) at 200 mU/mL in a special stabilizing solution. **CAUTION:** This material is isolated from human serum. Treat as potentially infectious.

225 µL Catalog Number C051-225UL

ThioStar® Detection Reagent
ThioStar thiol detection substrate stored in a ziploc pouch with desiccant. Reconstitute with dry DMSO.

2 vials Catalog Number C053-1EA

BChE Substrate
Butyrylthiocholine iodide freeze dried with stabilizers.

2 vials Catalog Number C052-1EA

Dry DMSO
Dry Dimethyl Sulfoxide solvent over molecular sieves. May be stored at room temperature.

14 mL Catalog Number X022-14ML

Assay Buffer Concentrate
A 10x concentrated Tris buffer containing detergents and stabilizers.

28 mL Catalog Number X064-28ML

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

DMSO, when stored at 4°C, will freeze. It can be stored tightly capped at room temperature.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm. Contact your plate reader manufacturer for correct filter sets. Set plate parameters for a 96-well Corning Costar 3650 plate. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

Software for converting raw relative fluorescent unit (FLU) 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.

Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals.

The Butyrylcholinesterase Standard is derived from human blood. It has been extensively tested for viral contamination, but all human blood products should be treated as potentially infectious and adequate precautions taken.

ThioStar® Detection Reagent should be stored at 4°C in the desiccated pouch. Allow desiccated pouch to warm to room temperature prior to opening. ThioStar will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate.

SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasmas from a variety of species. Samples containing visible particulate should be centrifuged prior to using. All samples and buffers should be free of excess thiols and reducing agents such as ß-mercaptoethanol, TCEP, or DTT.

SAMPLE PREPARATION

Serum & Plasma

Store separated serum or plasma on ice until assaying or freeze in aliquots for later use. Samples must be diluted in Assay Buffer prior to running in the kit. Any samples with BChE activity outside the standard curve range should be diluted further with Assay Buffer to obtain readings within the standard curve. Human serum and plasma typically have to be diluted ≥ 1:300 to read in the assay.

Use all samples within 2 hours of dilution.

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

Prepare the Assay Buffer by diluting one part of the 10x Assay Buffer Concentrate with nine parts deionized water for a 1:10 dilution. It is stable for up to 3 months when stored at 4°C.

ThioStar® Detection Reagent

Allow the ziploc pouch to warm **completely** to room temperature prior to opening. Remove a vial of ThioStar Reagent from the desiccator pouch and add 700 µL of the provided DMSO to the vial. Vortex thoroughly. This is a 10X concentrate of the ThioStar Detection Reagent. Seal any unused reconstituted Detection Reagent in the vial in the desiccator pouch. Store at 4°C and use within 2 months.

Butyrylcholinesterase Substrate

Add 700 µL of the provided DMSO to the BChE Substrate vial and vortex thoroughly. This is a 10x concentrate of the substrate. Store any unused reconstituted BChE Substrate in the vial at room temperature and use within 2 months.

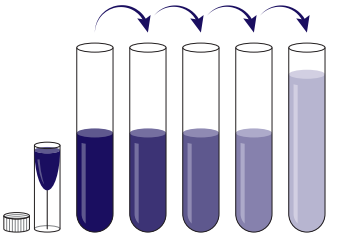
Reaction Mix Dilution Table

	1/2 Plate	Full Plate
10X BChE Substrate Concentrate	300 µL	550 µL
10X ThioStar® Concentrate	300 µL	550 µL
DMSO	2.4 mL	4.4 mL

REAGENT PREPARATION CONTINUED

Standard Preparation

BChE Standards are prepared by labeling test tubes as #1 through #7. Briefly spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #7. Carefully add 50 µL of the BChE Standard to tube #1 and vortex completely. Take 250 µL of the BChE solution in tube #1 and add it to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 through #7. The activity of BChE in tubes 1 through 7 will be 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mU/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)	**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 Conc. (mU/mL)	20	10	5	2.5	1.25	0.625	0.313

ASSAY PROTOCOL

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

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3650 plate.
   See: www.ArborAssays.com/resources/#general-info for plate dimension data.
2. Pipet 100 µL of samples or standards into duplicate wells in the plate.
3. Pipet 100 µL of Assay Buffer into duplicate wells as a Zero standard.
4. Add 50 µL of the prepared Reaction Mix to each of the wells using a repeater pipet.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 20 minutes.
7. Read the fluorescent emission at 510 nm with excitation at 370-410 nm. Please contact your plate reader manufacturer for suitable filter sets.

CALCULATION OF RESULTS

Average the duplicate FLU 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 FLUs for the zero standard. The sample activity obtained 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-butyrylcholinesterase-fluorescent-activity-kit.assay

BChE Unit Definition

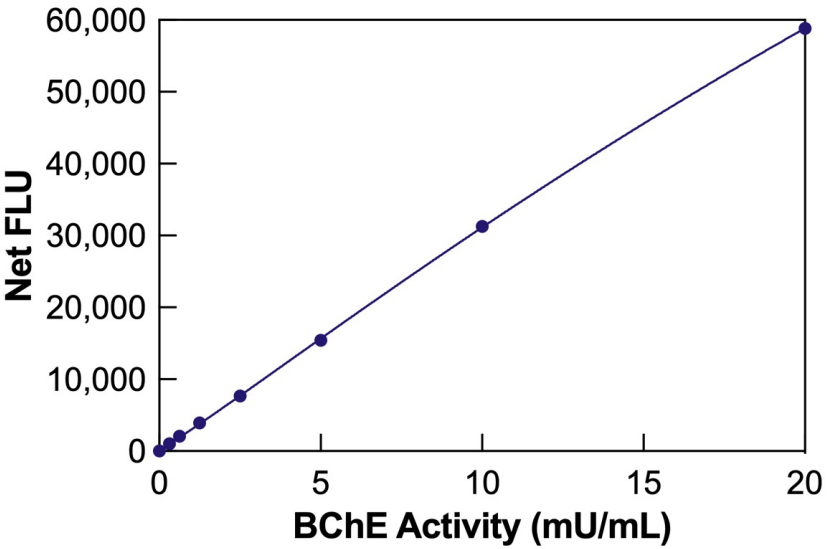
One unit will hydrolyze 1.0 µmol of butyrylcholine to choline and butyrate per minute at pH 8.0 and 37˚C.

TYPICAL DATA

Sample	Mean FLU	Net FLU	BChE Activity (mU/mL)
Standard 1	59,868	58,814	20
Standard 2	32,329	31,275	10
Standard 3	16,480	15,426	5
Standard 4	8,706	7,652	2.5
Standard 5	4,988	3,934	1.25
Standard 6	3,136	2,082	0.625
Standard 7	2,093	1,039	0.313
Zero	1,054	0	0
Sample 1	26,418	25,364	8.10
Sample 2	5,979	4,925	1.62

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

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 #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.018 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 and a low activity EDTA plasma sample. **The Limit of Detection was determined as 0.012 mU/mL**.

Intra Assay Precision

Three human serum samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated BChE activities were:

Sample	BChE Activity (mU/mL)	%CV
1	5.70	4.7
2	2.97	7.3
3	1.17	7.5

Inter Assay Precision

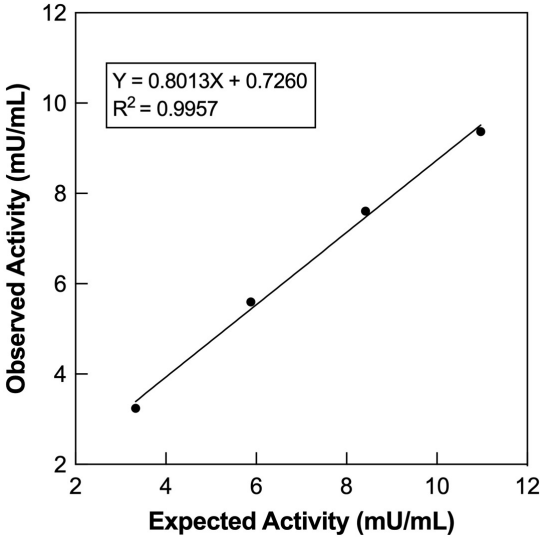
Three human serum samples were diluted with Assay Buffer and run in duplicates in sixteen assays run over multiple days by four operators. The mean and precision of the calculated BChE activities were:

Sample	BChE Activity (mU/mL)	%CV
1	7.70	9.1
2	5.84	7.5
3	1.71	8.5

Linearity

Linearity was determined by taking two plasma samples, one high sample diluted 1:450 and one low sample diluted 1:450, and mixing in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

Low Sample	High Sample	Expected Activity (mU/mL)	Observed Activity (mU/mL)	% Recovery
80%	20%	3.33	3.24	97.2
60%	40%	5.88	5.60	95.3
40%	60%	8.42	7.61	90.4
20%	80%	10.97	9.37	85.4
			Mean Recovery	**92.1%**

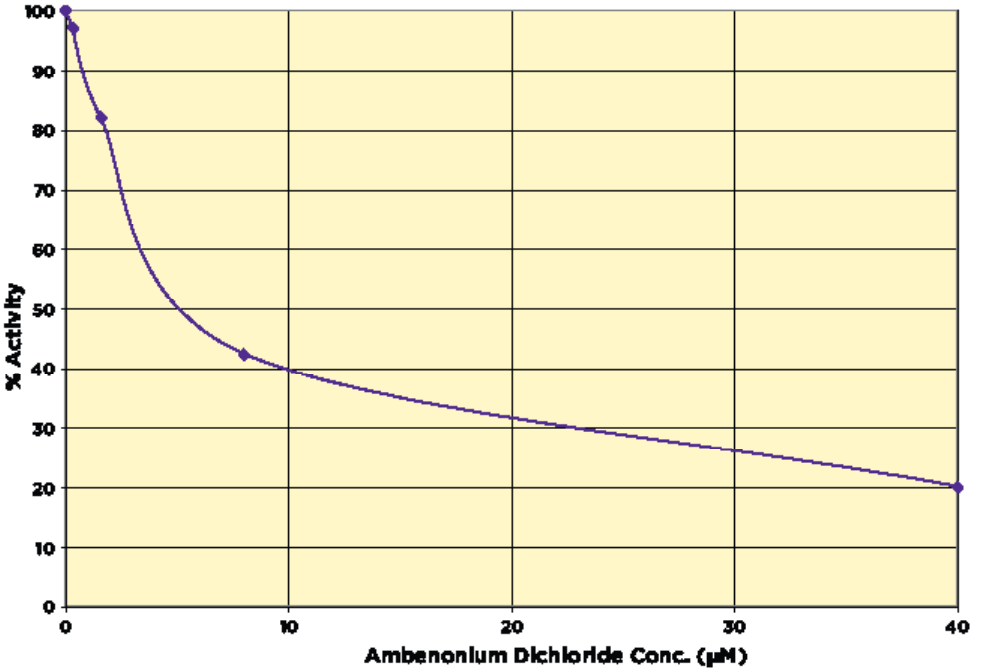


CROSS REACTIVITY

The assay was tested with a sample of human acetylcholinesterase from our DetectX® Acetylcholinesterase Fluorescent Activity Assay, Catalog Number K015-F1. The human AChE sample when used in the assay read at less than 0.2% of its expected activity.

INHIBITION STUDIES

The human BChE standard was incubated with varying concentrations of a reversible inhibitor of BChE activity, Ambenonium dichloride, from 200 µM down to 16 µM for 19 hours at room temperature in the kit Assay Buffer. The activity in the incubated enzyme samples was then determined. in the normal manner by adding 100 µL of the samples and reading the activity after a 20 minute incubation with 50 µL of Reaction Mixture.



SAMPLE VALUES

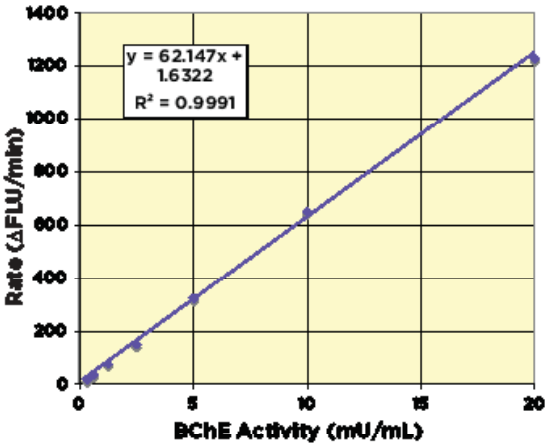
A variety of serum and plasma samples were tested in the assay, including chicken, mouse, rat, dog, monkey, pig and human samples. Values averaged 4,565 mU/mL. The average for 23 human serum and plasma samples was 6,268 ± 2,506 mU/mL. Five rat serum and plasma samples had low activity levels of between 293 and 365 mU/mL.

INTERFERENTS

A variety of additives were tested as possible interfering substances in the assay. Ethanol at 1% in the well decreased the activity recorded by 12.6%, whereas 0.5% in the well decreased activity by almost 10.3%. DMSO at 5% in the well increased activity by 0.2% and 1% in the well increased activity by 6.5%. Methanol at 10% in the well increased activity by 0.6%. Triton X-100 at 1% in the well increased activity 4.0% and hemoglobin at 0.1% decreased activity 4.2%. Controls should be run by the end user when appropriate.

END POINT VERSUS KINETIC ACTIVITY

A serum preparation diluted 1:900 read 11.89 mU/mL in Arbor Assays' endpoint assay. It was also read off a kinetic assessment of Butyrylcholinesterase activity and an activity of 12.37 mU/mL was obtained.



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

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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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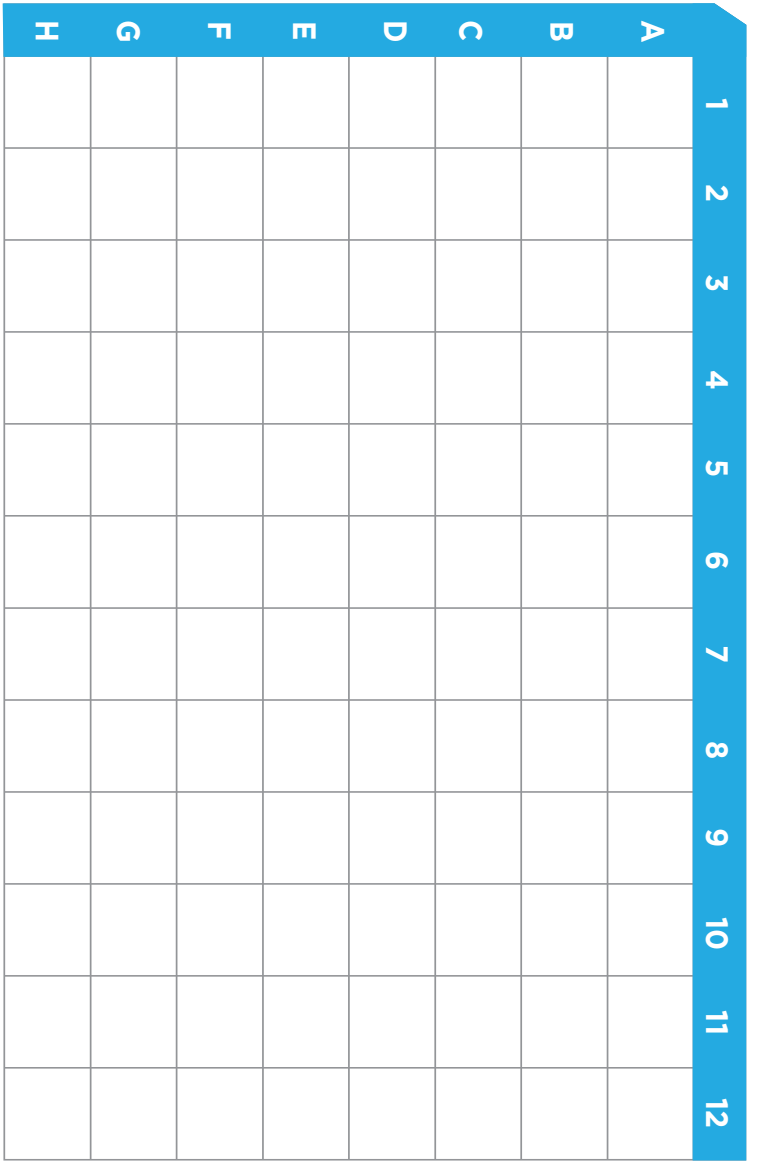

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