Butyrylcholinesterase Activity Assay Kit

Item No. 502895

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
400794	Assay Buffer A (5X)	1 vial/10 ml	4°C
401112	Enzyme Dilution Buffer	1 vial/6 ml	-20°C
401165	Butyrylcholinesterase Substrate	1 vial/800 µl	-20°C
760912	DTNB Assay Reagent	2 vials	-20°C
401098	BChE (human, recombinant)	1 vial	-20°C
401099	Cholinesterase Inhibitor	1 vial/50 µl	-20°C
401101	Acetylcholinesterase Inhibitor	1 vial/50 µl	-20°C
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Butyrylcholinesterase Activity Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 410 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled water is acceptable. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 10)

INTRODUCTION

Background

Butyrylcholinesterase (BChE) is a highly conserved carboxylesterase.[1] It is widely expressed in the periphery and brain and can hydrolyze butyrylthiocholine, acetylcholine, and certain ester-containing exogenous substances.[1-3] BChE is involved in the regulation of the peptide hormone ghrelin and is found at reduced levels in the blood of infants with sudden infant death syndrome (SIDS).[4] In addition, loss-of-function mutations in *BCHE* are associated with the accumulation of exogenous BChE substrates, including the muscle relaxant succinylcholine, which can lead to adverse effects such as prolonged apnea.[3,5]

BChE and a similar carboxylesterase, acetylcholinesterase (AChE), arose from a common ancestral precursor.[6] Both enzymes are expressed in certain tissues, such as the brain, but the patterns of expression and overlap vary based on cell type and region. BChE and AChE are also both found in the serum, where AChE is present at very low levels in humans but at higher levels in certain mammals, such as cows and horses. Both BChE and AChE are implicated in the pathophysiology of Alzheimer's disease.[7,8] Interestingly, *Bche* knockout mice have a normal phenotype, apart from an impairment in the inactivation of succinylcholine, and BChE can partially compensate for AChE in the *Ache* knockout mouse.[6] This compensation and the overlap in expression and activity makes differentiating the roles of AChE and BChE important for the development of therapeutics for these targets.

About This Assay

Cayman's Butyrylcholinesterase Activity Assay Kit provides a convenient colorimetric method for measuring BChE activity in serum, plasma, tissue homogenates, and cell lysates. In the assay, BChE hydrolyzes butyrylthiocholine to generate thiocholine, which reacts with DTNB to produce a yellow-colored product with strong absorbance at 410 nm. Non-specific activity from AChE is inhibited by the AChE inhibitor provided. A general cholinesterase (ChE) inhibitor is also included for use in sample background controls.

This assay has a limit of detection (LOD) of 0.5 mU/ml and a lower limit of quantification (LLOQ) of 1.0 mU/ml

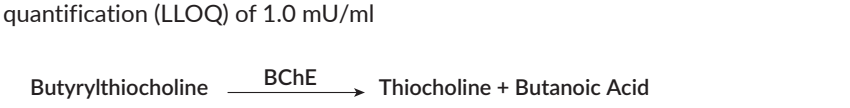

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Assay Buffer A (5X) – (Item No. 400794)**

   This vial contains 10 ml of Assay Buffer A (5X). Dilute the contents of the vial with 40 ml pure water to make the assay buffer. Rinse the vial to remove any salts that may have precipitated. The assay buffer will be stable for two months when stored at 4°C.

   NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with pure water.

2. **Butyrylcholinesterase Substrate – (Item No. 401165)**

   This vial contains 800 µl of Butyrylcholinesterase Substrate. If not using all at once, prepare aliquots and store at -20°C, where it will be stable for two months. Limit freeze-thaw cycles to three.

   Prior to use in the assay, dilute 600 µl of the substrate with 11.4 ml of assay buffer. This is a sufficient volume to assay 100 wells. Scale as needed. The diluted substrate will be stable for two hours on ice.

3. **DTNB Assay Reagent – (Item No. 760912)**

   Each vial contains lyophilized DTNB. Reconstitute the contents of the vial with 1 ml of pure water and place on ice. Store on ice, protected from light. Use the reconstituted DTNB Assay Reagent within the same day.

   Prior to use in the assay, dilute the reconstituted DTNB Assay Reagent with 9 ml of assay buffer. This is a sufficient volume to assay 100 wells. Scale as needed. The diluted reagent will be stable for two hours on ice.

4. **Enzyme Dilution Buffer - (Item No. 401112)**

   This vial contains 6 ml of a dilution buffer formulated for recombinant BChE. Biological samples should be diluted in assay buffer, not Enzyme Dilution Buffer.

5. **BChE (human, recombinant) - (Item No. 401098)**

   This vial contains lyophilized BChE (human, recombinant) to be used as a positive control. Reconstitute the contents of the vial with 250 µl of assay buffer and place on ice. *Mix gently; do not vortex.* If not using all at once, prepare aliquots and store at -80°C, where it will be stable for three weeks. Limit freeze-thaw cycles to three.

   Prior to use in the assay, dilute 20 µl of the reconstituted BChE enzyme with 180 µl of Enzyme Dilution Buffer. The diluted enzyme will be stable for two hours on ice.

6. **Cholinesterase Inhibitor - (Item No. 401099)**

   This vial contains 50 µl of Cholinesterase Inhibitor, which inhibits both AChE and BChE and is used in sample background reactions. If not using all at once, prepare aliquots and store at -20°C. Limit freeze-thaw cycles to three.

   Prior to use in the assay, dilute 5 µl with 495 µl of assay buffer. This is a sufficient volume to assay 20 wells. The diluted inhibitor will be stable for four hours on ice.

7. **Acetylcholinesterase Inhibitor - (Item No. 401101)**

   This vial contains 50 µl of an AChE-specific inhibitor. If not using all at once, aliquot and store at -20°C. Prior to use in the assay, dilute 5 µl with 495 µl of assay buffer. This is a sufficient volume to assay 20 wells. The diluted inhibitor will be stable for four hours on ice.

Sample Preparation

Samples should be processed and assayed immediately after collection; samples that cannot be assayed immediately should be flash frozen and stored at -80°C. To normalize BChE activity, the concentration of total protein in each sample must be determined. *Protein Determination Kits (Item Nos. 701780 and 760200) are available for purchase from Cayman.*

Endogenous BChE exhibits a broad range of activity across samples. To ensure that readings fall within the range of the assay, samples may require dilution in assay buffer. Testing multiple dilutions is recommended to verify linearity and obtain accurate, reproducible measurements. Suggested dilution ranges are provided below.

Plasma

1. Collect blood in vacutainers containing heparin or EDTA as an anticoagulant.
2. Centrifuge at 1,000 x g for 15 minutes at 4°C.
3. Transfer the top plasma layer into a clean test tube without disturbing the white buffy layer.
4. Dilute with assay buffer; typical dilutions range from 1:20 to 1:160.

Serum

1. Collect blood in vacutainers without an anticoagulant.
2. Allow samples to clot undisturbed for 30-60 minutes at room temperature.
3. Centrifuge at 1,000-2,000 x g for 15-30 minutes at 4°C.
4. Transfer the top serum layer into a clean test tube.
5. Dilute with assay buffer; typical dilutions range from 1:20 to 1:160.

Tissue Homogenate

1. Prior to dissection, rinse the tissue with PBS, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 0.5 ml of cold assay buffer per 50 mg of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Transfer the supernatant to a new tube and discard the pellet.
5. Optional: determine total protein concentration to normalize activity across samples; alternatively, normalize to tissue mass.

NOTE: BChE activity can vary greatly between tissue types. To ensure readings fall within the assay's linear range, multiple sample dilutions should be tested.

Cell Lysate

1. Pellet cells (~5 x 10^6 cells) by centrifugation (*i.e.* 1,000-2,000 x g for 5 minutes at 4°C). For adherent cells, harvest using a cell scraper instead of proteolytic enzymes.
2. Homogenize the cell pellet in 0.2 ml cold assay buffer.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Transfer the supernatant to a new tube and discard the pellet.
5. Optional: determine total protein concentration to normalize activity across samples; alternatively, normalize to cell number.

NOTE: Cell lysates typically have high background; however, dilution is generally not needed as background absorbance is subtracted when calculating BChE activity.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each assay contain positive control wells, sample wells, and sample background wells. A typical layout in duplicate is shown in Figure 2 below. It is suggested that the contents of each well be recorded on the template sheet provided (see page 26).

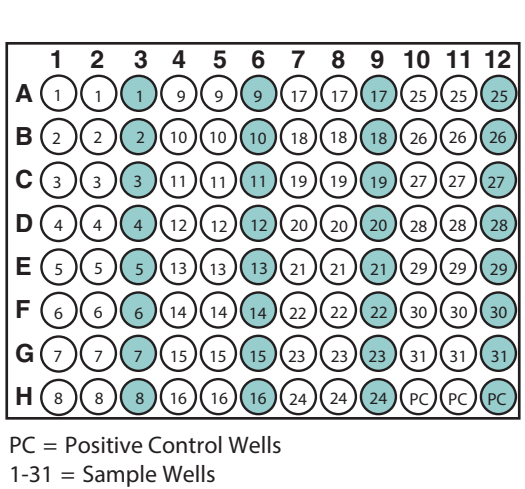

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all of the wells.
- The volume of sample needed per well is 20 µl.
- All diluted reagents, except for the assay buffer and Enzyme Dilution Buffer should be kept on ice before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- The assay is performed at room temperature (22-25°C).
- Read the absorbance at 410 nm.

Performing the Assay

1. Add the reagents to the designated wells according to Table 1, below. Mix thoroughly and ensure no bubbles are formed. *NOTE: Sample background wells are used to correct for non-specific signal from endogenous free thiols in the sample.*

Reagent	Positive Control Wells	Sample Wells	Sample Background Wells
Diluted DTNB	80 µl	80 µl	80 µl
Assay Buffer	20 µl	--	--
Sample	--	20 µl	20 µl
Diluted BChE	20 µl	--	--
Diluted AChE Inhibitor	--	20 µl	--
Diluted ChE Inhibitor	--	--	20 µl

Table 1. Pipetting summary

2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 15 minutes at room temperature, protected from light.
3. Remove the plate cover and quickly initiate the reactions by adding 80 µl of the diluted Butyrylcholinesterase Substrate to all wells. Mix thoroughly and ensure no bubbles are formed.
4. Immediately measure absorbance at 410 nm once every minute for 30 minutes at room temperature. If BChE activity is low, continue reading for up to 60 minutes.

ANALYSIS

Calculations

1. Determine the change in absorbance per minute (ΔA_{410}/min) for each sample, positive control, and sample background well by plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve (see Figure 4, page 19).
2. If the average sample background rate is >0, subtract this value from the average rates of each sample.
3. Calculate enzyme activity (U/ml) using the equation below. One unit is defined as 1 µmol of substrate hydrolyzed per minute under the specified conditions of this assay.

$$\text{BChE activity (U/ml)} = \left[\frac{\Delta A_{410}/\text{min}}{9.34\ \text{mM}^{-1}}\right] \text{X} \frac{200\ \mu\text{l}}{20\ \mu\text{l}} \text{X DF}$$

Where:

- 9.34 mM^{-1}: extinction coefficient that has been adjusted for the pathlength of the solution in the well.
- 200 µl: volume of total reaction
- 20 µl: volume of sample used in reaction
- DF: dilution factor of sample

4. Optional: BChE activity can be normalized to total protein concentration:

$$\text{Normalized BChE activity (U/mg)} = \left[\frac{\text{BChE activity (U/ml)}}{\text{protein (mg/ml)}}\right]$$

Example Data

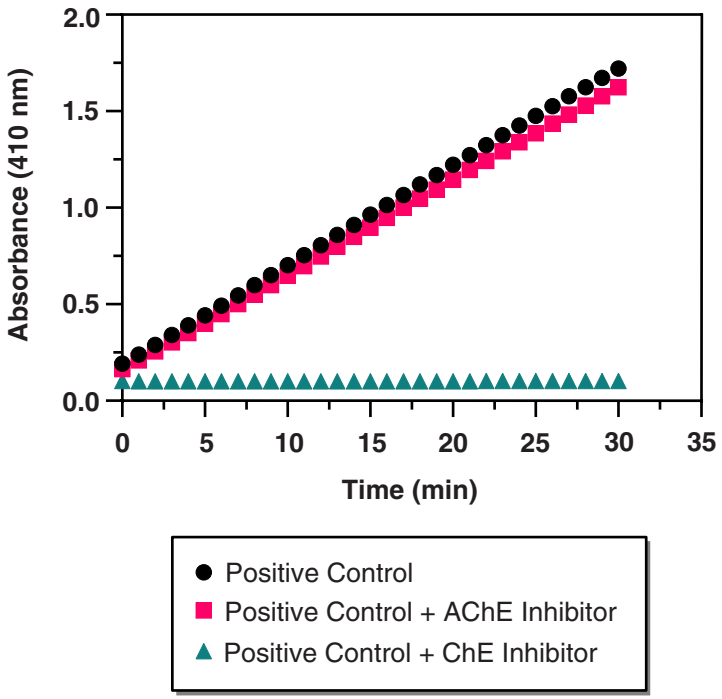

Figure 3. Inhibition of the BChE positive control by the ChE inhibitor

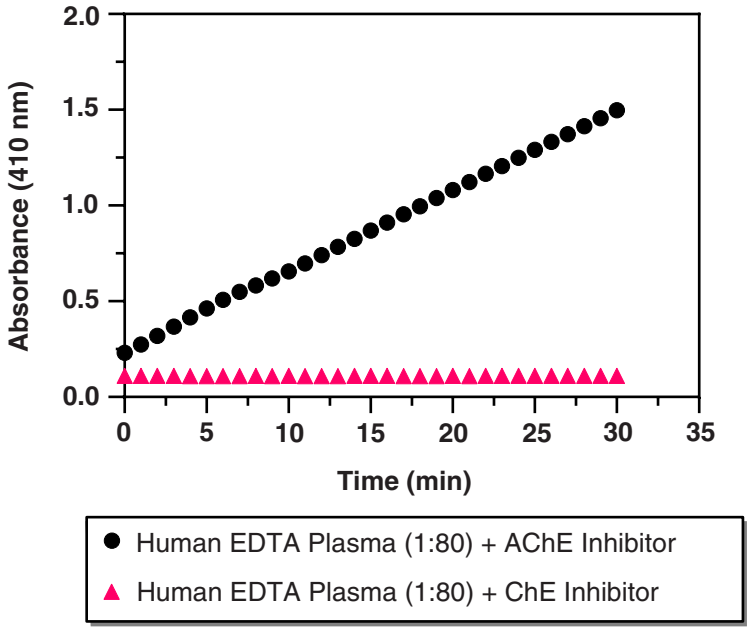

Figure 4. Absorbance *versus* time for human EDTA plasma (1:80) in the presence of AChE or ChE inhibitors

Interferences

Interference was evaluated by preparing the positive control in the presence of the indicated reagents at specified concentrations. A reagent is considered to interfere with the assay if it results in: (1) 10% difference in expected activity; or (2) non-linearity of BChE activity on dilution.

Reagents		Will Interfere?
Buffers	M-PER (≤20%)	No
	T-PER (≤20%)	No
	RIPA (≤20%)	No
Detergents	SDS (≥0.01%)	Yes
	Triton X-100 (≥0.1%)	Yes
	Tergitol (≤1%)	No
	Polysorbate 20 (≥1%)	Yes
Chelators	EDTA (≤1.6 mM)	No
Protease Inhibitors/ Enzymes	Aprotinin (≥0.06 µg/ml)	Yes
	Bestatin (≥40 µg/ml)	Yes
	E-64 (≥0.5 µg/ml)	Yes
	Leupeptin, Microbial (≥10 µM)	Yes
	Pepstatin A (≥0.7 µg/ml)	Yes
	AEBSF (hydrochloride) (≥100 µM)	Yes
Solvents	DMSO (≥4 %)	Yes
Others	BSA (≤0.2 %)	No
	Glycerol (≤2%)	No
	Sucrose (≤2%)	No
	Triglyceride (Intralipid) (≤160 mg/dl)	No
	Hemoglobin (≤200 mg/dl)	No
	Bilirubin (≥2.4 mg/dl)	Yes
	Bilirubin conjugate (≤10 mg/dl)	No

Table 2. Interferences

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate to remove bubbles
No activity detected in positive control and sample wells	A. Substrate was not added to the wells B. The ChE inhibitor was added to the wells	Re-assay with the proper components added to the wells
No signal increase over time observed in the samples	A. BChE activity is too low to detect B. There is no BChE in the sample C. If the signal at t_0 is much higher than the background signal, the reaction has already reached completion; the enzyme level is too high	A. Re-assay using a lower dilution B. Verify expression and re-assay C. Dilute the sample further and re-assay
No signal increase over time in positive control wells	A. Positive control is inactive B. Positive control was not added to the well	A. Verify proper storage and handling; prepare a fresh batch of positive control and re-assay B. Make sure to add all the components to the wells and re-assay

References

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2. Patocka, J., Kuca, K., and Jun, D. Acetylcholinesterase and butyrylcholinesterase--important enzymes of human body. *Acta Medica (Hradec Kralove)* **47(4)**, 215-228 (2004).
3. Yen, T., Nightingale, B.N., Burns, J.C., *et al.* Butyrylcholinesterase (BCHE) genotyping for post-succinylcholine apnea in an Australian population. *Clin. Chem.* **49(8)**, 1297-1308 (2003).
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5. Andersson, M.L., Møller, A.M. and Wildgaard, K. Butyrylcholinesterase deficiency and its clinical importance in anaesthesia: A systematic review. *Anaesthesia* **74(4)**, 518-528 (2019).
6. Mushtaq, G., Greig, N.H., Khan, J.A., *et al.* Status of acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease and type 2 diabetes mellitus. *CNS Neurol. Disord. Drug Target.* **13(8)**, 1432-1439 (2014).
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Assay Summary

Item No.	Reagent	Procedure
400794	Assay Buffer A (5X)	Dilute 1:5 using pure water
401165	Butyrylcholinesterase Substrate	Dilute 1:20 using assay buffer prior to use
760912	DTNB Assay Reagent	Reconstitute one vial with 1 ml pure water; dilute 1:10 using assay buffer prior to use
401112	Enzyme Dilution Buffer	Thaw; ready to use
401098	BChE (human, recombinant)	Reconstitute with 250 µl assay buffer; dilute 1:10 using Enzyme Dilution Buffer before use
401099	ChE Inhibitor	Dilute 1:100 using assay buffer
401101	Acetylcholinesterase Inhibitor	Dilute 1:100 using assay buffer

Table 3. Reagent preparation summary

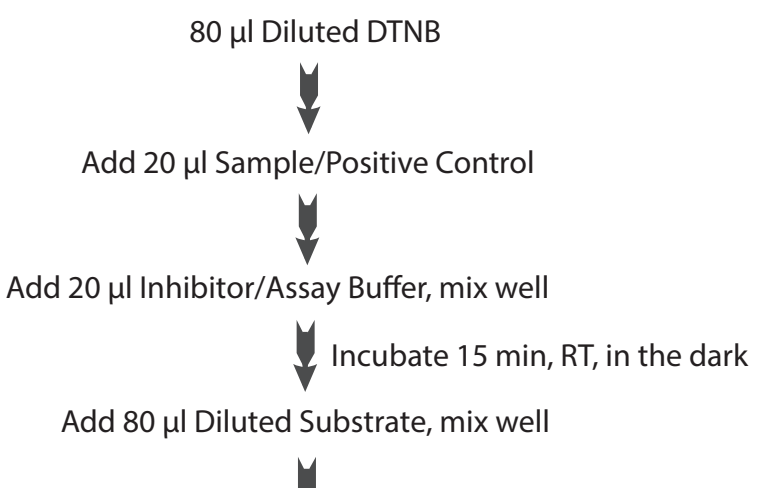
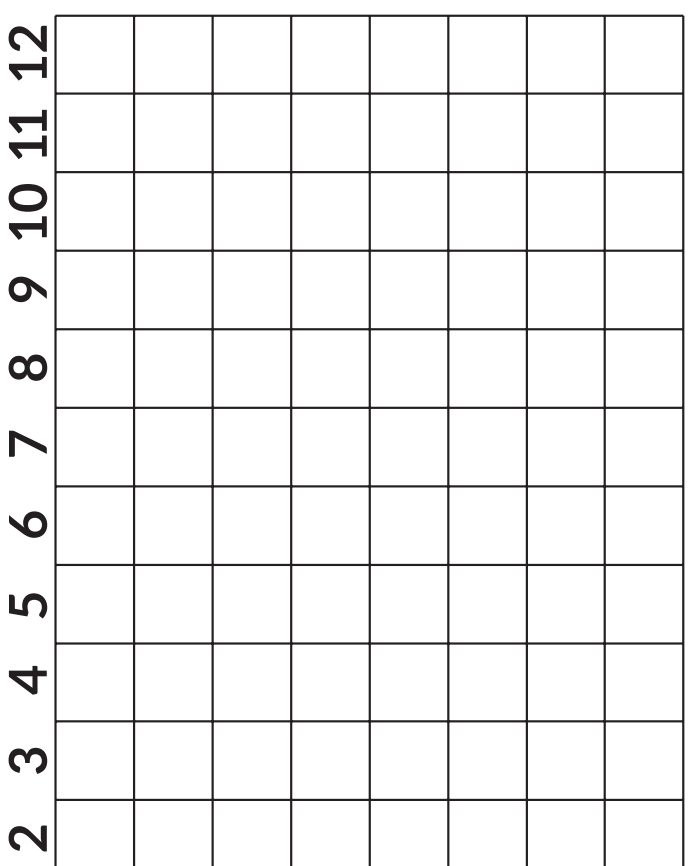


Figure 5. Assay summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.