TBARS Assay Kit

Item No. 10009055

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Customer Service 800.364.9897
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TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity	Storage
700871	Thiobarbituric Acid Assay Reagent	1 vial/1 g	RT
700872	TBA Acetic Acid	1 vial/10 ml	RT
700017	Sodium Hydroxide Assay Reagent (3.5 M)	1 vial/10 ml	RT
10009202	TBA Malondialdehyde Standard	1 vial/1 ml	4°C
10009203	TBA SDS Solution	1 vial/10 ml	4°C
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	RT
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	2 covers	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.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.), as some of them may be harmful.

The sodium hydroxide and acid solutions are corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

Care should be exercised when removing samples from boiling water.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3640

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 at 4°C 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 between 530-540 nm or a fluorometer with the capacity to measure fluorescence using an excitation wavelength of 530 nm and an emission wavelength of 550 nm with bandwidths set to 10 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. A dry bath that heats up to 95°C or a container suitable for boiling samples and standards
5. 1.5 ml centrifuge tubes with a lock or 2 ml centrifuge tubes with screw caps
6. Centrifuge capable of spinning 1.5-2 ml centrifuge tubes at 1,600 x g
7. Materials used for **Sample Preparation** (see page 9)

Alternate Assay

Cayman Chemical also offers an alternative assay kit for TBARS assessment (TBARS (TCA Method) Assay Kit; Item No. 700870). While maintaining the reliability and accuracy of the of the original assay, this assay offers the advantage of an improved sample processing resulting from the formation of harder protein pellets during TCA precipitation.

INTRODUCTION

Background

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues.[1,2] Lipid peroxides, derived from PUFAs, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, such as MDA. In human platelets, thromboxane synthase also catalyzes the conversion of PGH_2 to thromboxane A_2, 12(S)-HHTrE, and MDA in a ratio of 1:1:1.[3]

The measurement of Thiobarbituric Acid Reactive Substances (TBARS) is a well-established method for screening and monitoring lipid peroxidation.[1,2] Modifications of the TBARS assay by many researchers have been used to evaluate several types of samples including human and animal tissues and fluids, drugs, and foods.[4-8] Even though there remains a controversy cited in literature regarding the specificity of TBARS toward compounds other than MDA, it still remains the most widely employed assay used to determine lipid peroxidation.[2] If lipoprotein fractions are first acid precipitated from the sample, interfering soluble TBARS are minimized, and the test becomes quite specific for lipid peroxidation.[2] Lipids with greater unsaturation will yield higher TBARS values. It is recommended that if high TBARS values are obtained, a more specific assay such as HPLC should be performed.

About This Assay

Cayman's TBARS Assay Kit provides a simple, reproducible, and standardized tool for assaying lipid peroxidation in plasma, serum, urine, tissue homogenates, and whole cell lysates. The MDA-TBA adduct formed by the reaction of MDA and TBA under high temperature (90-100°C) and acidic conditions is measured colorimetrically at 530-540 nm or fluorometrically at an excitation wavelength of 530 nm and an emission wavelength of 550 nm (see Figure 1 below). Although this reaction has a much higher sensitivity when measured fluorometrically, protocols for both methods are provided. When read fluorometrically, this assay has a range of 0.0625-5 µM of MDA equivalents and when read colorimetrically, the assay range is 0.625-50 µM of MDA equivalents.

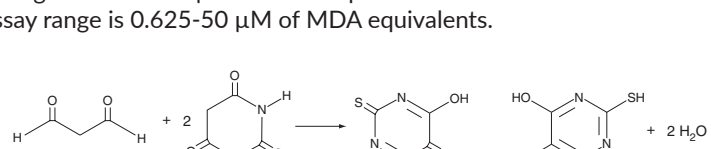

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Thiobarbituric Acid Assay Reagent - (Item No. 700871)**

   The vial contains 1 g of thiobarbituric acid (TBA). It is ready to use to prepare the Color Reagent.

2. **TBA Acetic Acid - (Item No. 700872)**

   Each vial contains 10 ml of concentrated acetic acid. Slowly add TBA Acetic Acid to 40 ml of HPLC-grade water. This diluted Acetic Acid Solution is used in preparing the Color Reagent and it is stable for at least three months at room temperature.

3. **Sodium Hydroxide Assay Reagent (3.5 M) - (Item No. 700017)**

   The vial contains a solution of sodium hydroxide (NaOH). Dilute 10 ml of TBA NaOH Assay Reagent with 40 ml of HPLC-grade water. This diluted NaOH Solution is used in preparing the Color Reagent. The diluted NaOH Solution is stable for at least three months at room temperature. Store the diluted NaOH Solution in a plastic container suitable for corrosive materials.

4. **TBA Malondialdehyde Standard - (Item No. 10009202)**

   The vial contains 500 µM malondialdehyde (MDA) in water. It is ready to use to prepare the standard curve.

5. **TBA SDS Solution - (Item No. 10009203)**

   The vial contains a solution of sodium dodecyl sulfate (SDS). The solution is ready to use as supplied.

6. **To prepare the Color Reagent:**

   The following amount of Color Reagent is sufficient to evaluate 24 samples. Adjust the volumes accordingly if more or less samples are going to be assayed. Weigh 106 mg of TBA (Item No. 700871) and add to a beaker containing 10 ml of diluted TBA Acetic Acid Solution. Add 10 ml of diluted TBA Sodium Hydroxide and mix until the TBA is completely dissolved. The solution is stable for 24 hours at 4°C.

Sample Preparation

Plasma

Typically, normal human plasma has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 µM.[1,8]

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

Serum

Typically, normal human serum has a lipid peroxide level (expressed in terms of MDA) of 1.86-3.94 µM.[1]

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Serum does not need to be diluted before assaying.

Urine

Typically, normal human urine has a lipid peroxide level (expressed in terms of MDA) of 0.8-2 µmol/g creatinine.[9,10]

1. Urine does not require any special treatments. If not assaying the same day, freeze at -80°C.

Tissue Homogenates

1. Weigh out approximately 25 mg of tissue into a 1.5 ml centrifuge tube.
2. Add 250 µl of RIPA Buffer containing protease inhibitors of choice (see **Interferences** section on page 22). *NOTE: RIPA buffer is available for purchase from Cayman (Item No. 10010263)*
3. Homogenize or sonicate the tissue on ice.
4. Centrifuge the tube at 1,600 x g for 10 minutes at 4°C. Use the supernatant for analysis. Store supernatant on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for one month.
5. Tissue homogenates do not need to be diluted before assaying.

NOTE: For tissue samples it is necessary to prepare the standard curve in the same RIPA buffer that is used to prepare the samples.

Whole Cell Lysates

1. Collect 1-2 x 10^7 cells in 1 ml of buffer of choice, such as PBS.
2. Homogenize or sonicate the cells on ice.
3. Use the whole cell lysate in the assay.
4. Cell lysates do not need to be diluted before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of standards and samples to be measured in duplicate is shown below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 25).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H = Standard Wells
S1-S40 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- 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

- All reagents, except samples, must be equilibrated to room temperature before beginning the assay. The SDS Solution will take at least one hour to equilibrate to room temperature if stored at 2-8°C. Briefly heating the SDS Solution at 37°C will re-dissolve the precipitated SDS. The SDS Solution can then be stored at room temperature.
- The final volume of the assay is 200 µl in all wells.
- The assay is performed at room temperature, except for the heating/boiling step which is performed at 95-100°C.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples and standards be assayed at least in duplicate.
- Monitor the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm. For the fluorometric determination, it is recommended that the excitation and emission bandwidths are set to 10 nm.

Colorimetric Standard Preparation

Dilute 250 µl of the MDA Standard (Item No. 10009202) with 750 µl of water to obtain a stock solution of 125 µM. Take eight clean glass test tubes or polypropylene tubes and label them A-H. Add the amount of 125 µM MDA stock solution and water to each tube as described in Table 1. *NOTE: If assaying tissue samples, the same RIPA buffer that was used to prepare samples should be used in place of water for stock standard and standard curve preparation.*

Tube	MDA Stock Solution (µl)	Water (µl)	MDA Concentration (µM)
A	0	1,000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

Table 1. MDA colorimetric standards

Fluorometric Standard Preparation

Dilute 25 µl of the MDA Standard (Item No. 10009202) with 975 µl of water to obtain a stock solution of 12.5 µM. Take eight clean glass test tubes or polypropylene tubes and label them A-H. Add the amount of 12.5 µM MDA stock solution and water to each tube as described in Table 2. *NOTE: If assaying tissue samples, the same RIPA buffer that was used to prepare samples should be used in place of water for stock standard and standard curve preparation.*

Tube	MDA Stock Solution (µl)	Water (µl)	MDA Concentration (µM)
A	0	1,000	0
B	5	995	0.0625
C	10	990	0.125
D	20	980	0.25
E	40	960	0.5
F	80	920	1
G	200	800	2.5
H	400	600	5

Table 2. MDA fluorometric standards

Performing the Assay

1. Add 100 µl of sample or standard to appropriately labeled, 1.5 ml centrifuge tube with a lock, or a similar size vial with a screw cap.
2. Add 100 µl of SDS Solution to each tube and swirl to mix.
3. Add 800 µl of the Color Reagent.
4. Cap tubes tightly and heat samples at 95°C for 1 hour in a dry bath. (Alternatively, samples can be boiled for 1 hour. Make sure the samples are held upright during boiling).
5. Immediately remove the tubes and place in an ice bath to stop the reaction. Incubate on ice for 5 minutes.
6. Centrifuge the vials for 10 minutes at 1,600 x g at room temperature. The solutions may appear clear or cloudy. Cloudiness will clear upon warming to room temperature. Processed samples and standards are stable at room temperature for 30 minutes.
7. Transfer 200 µl from each tube to either the clear plate (colorimetric assay) or black plate (fluorometric assay). Be careful not to disturb the pellet at the bottom of the tube.
8. Read the absorbance at 530-540 nm or read fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 550 nm with the excitation and emission bandwiths set no higher than 10 nm.

ANALYSIS

Calculations

1. Calculate the average absorbance or fluorescence value of each standard and sample.
2. Subtract the absorbance or fluorescence value of the standard A (0 µM) from itself and all other values (both standards and samples). This is the corrected signal value (CSV).
3. Plot the corrected signal values (from step 2 above) of each standard as a function of MDA concentration (see Tables 1 and 2, on pages 13 and 14).
4. Calculate the concentration of MDA for each sample from the standard curve. Examples of the MDA standard curves are shown on pages 17-18 in Figures 3 and 4.

$$\text{MDA (µM)} = \left[\frac{\text{(CSV) - (y-intercept)}}{\text{Slope}}\right]$$

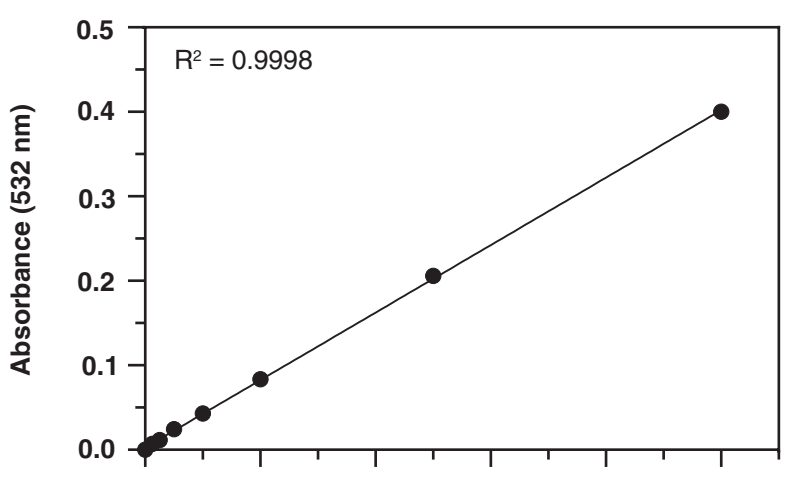

Figure 3. MDA colorimetric standard curve

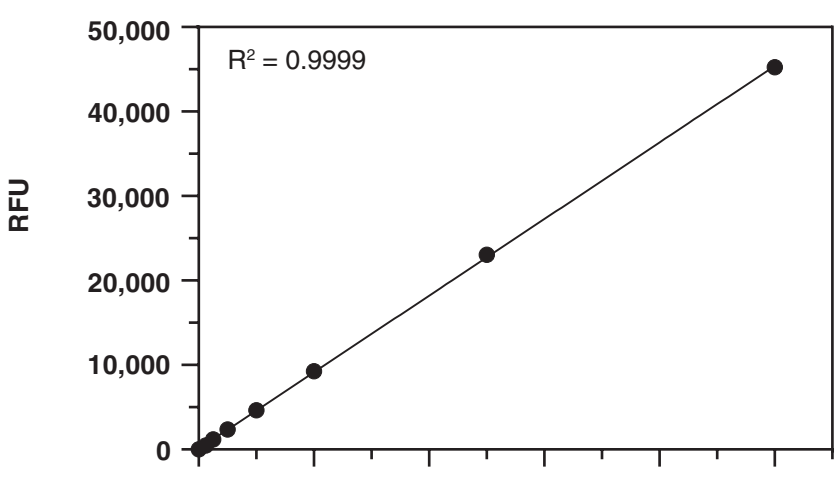

Figure 4. MDA fluorometric standard curve

Performance Characteristics

Precision:

When a series of five human serum samples were assayed on the same day, the intra assay coefficient of variation was 8% and 5% for colorimetric and fluorometric assays, respectively. When three urine and three serum samples were tested on different days, the inter-assay coefficient of variation was 1% and 12%, respectively for colorimetric assay and 13% and 3%, respectively for fluorometric assay.

Spike and Recovery:

Human urine and HepG2 whole cell lysate were spiked with different amounts of MDA and evaluated using TBARS assay kit.

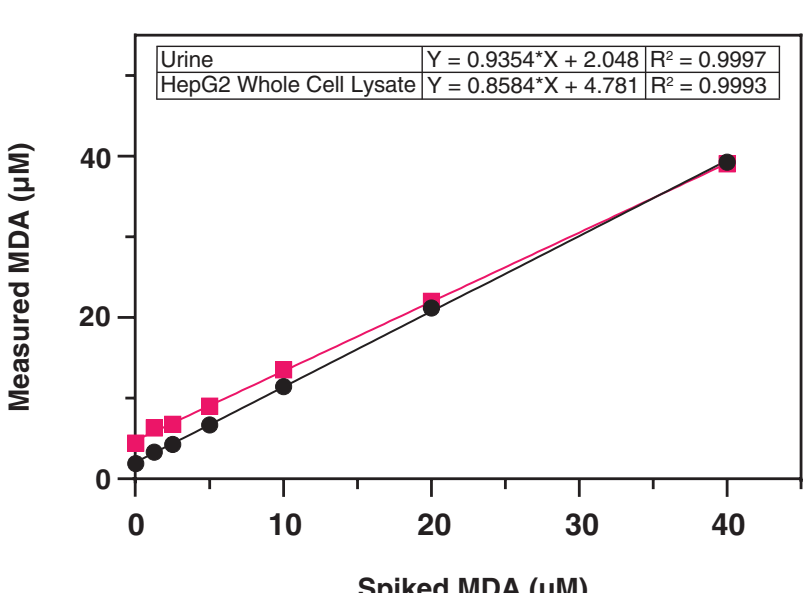

Figure 5. Spike and recovery of MDA in human urine and HepG2 whole cell lysate in colorimetric assay format

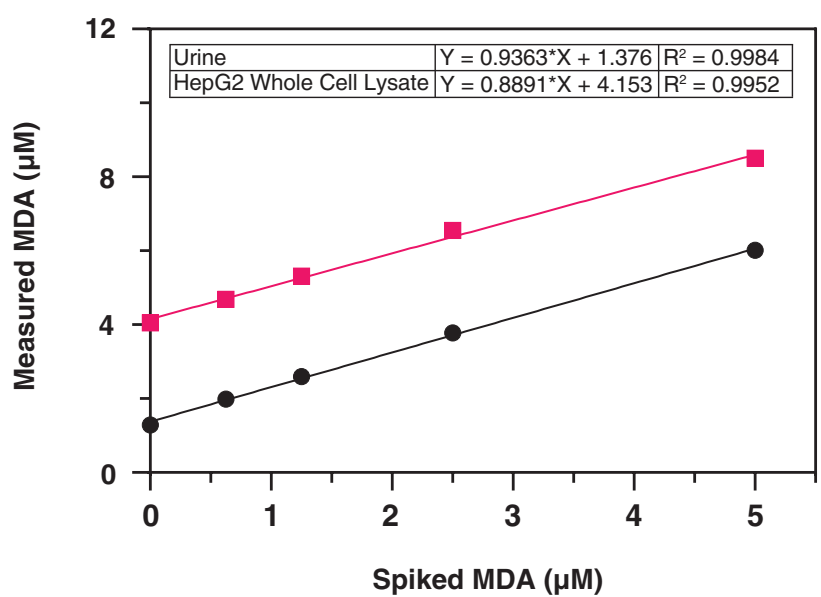

Figure 6. Spike and recovery of MDA in human urine and HepG2 whole cell lysate in fluorometric assay format

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

	Reagent	Will Interfere (Yes or No)
Buffers:	Borate (50 mM)	No
	HEPES (100 mM)	No
	Phosphate (100 mM)	No
	Tris (25 mM)	No
	RIPA	Yes
Detergents:	CHAPS (≤1%)	No
	Triton X-100 (≤1%)	No
	Polysorbate 20 (≤1%)	No
Protease Inhibitors/Chelators:	Antipain (≤0.1 mg/ml)	No
	Chymostatin (≤10 µg/ml)	No
	Leupeptin (≤10 µg/ml)	No
	PMSF (≤200 µM)	No
	Trypsin (≤10 µg/ml)	No
	EDTA (≤1 mM)	No
	EGTA (≤1 mM)	No
	Pepstatin (0.7 µg/ml)	No
	Phosphoramidon (330 µg/ml)	No
	Pefabloc SC (1 mg/ml)	No
	Aprotinin (2 µg/ml)	No
	E-64 (10 µg/ml)	No
	Bestatin (40 µg/ml)	No
Others:	Sucrose (250 mM)	Yes
	Glycerol (≤10%)	No

Table 3. Interferences

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/ technique B. Bubbles in the well(s) C. Sample evaporated during heating D. Bandwidths are too high	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles C. Use tubes that seal tightly D. Set bandwidths in florometer to ≤ 10 nm and re-read
No MDA was detected in the sample	A. MDA concentration was too low B. The sample was too dilute	A. Process more tissue (50-100 mg) and re-assay B. Harvest more cells (2×10^8) and re-assay
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read

References

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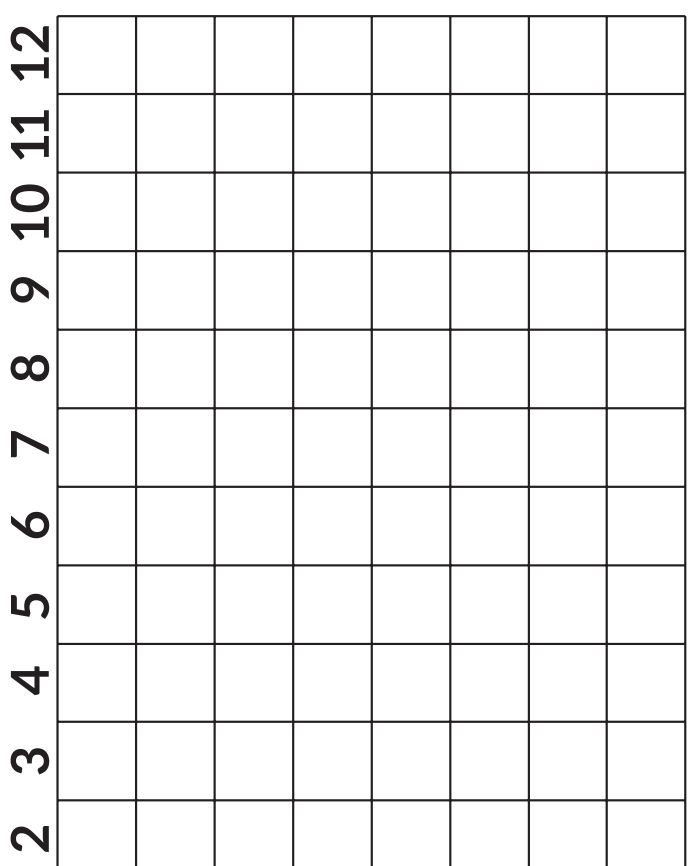

NOTES

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.