Prostaglandin D_2 FPIA Kit - Red

Item No. 512051

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Materials Supplied

Item Number	Item	96 wells/ 384 wells Quantity/Size	480 wells/ 1,920 wells Quantity/Size
412050	Prostaglandin D_2 FPIA Reagent - Red	1 vial/400 dtn	1 vial/2,000 dtn
412054	Prostaglandin D_2 FPIA Standard - Red	1 vial/500 µl	1 vial/500 µl
400501	FPIA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
10005372/10005371	96-Well Plate (black); 384-Well Plate (black)	1 plate	5 plates
400023	Foil Plate Cover	1 cover	5 covers

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with the Cayman Prostaglandin D_2 FPIA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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 at -80°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 fluorescence polarization measurements using rhodamine as the fluorophore.
2. Adjustable pipettes and a repeat pipettor.

INTRODUCTION

Background

Prostaglandin D_2 (PGD_2) is biosynthesized in the brain by a soluble, 26 kD glutathione-independent lipocalin-type PGD_2 synthase (L-PGDS).[1] PGD_2 accumulates in the cerebrospinal fluid (CSF), where it induces physiologic sleep in rats and humans.[2] PGD_2 is also synthesized by mast cells and leukocytes by a cellular, myeloid-type, glutathione-dependent PGD synthase (hematopoietic PGD synthase; H-PGDS).[3] This PGD_2 which is formed in the intracellular and vascular compartments is rapidly metabolized to 11β-$PGF_{2\alpha}$ as well as a variety of β-oxidation products including tetranor-PGDM.[5] Measurement of the parent eicosanoid PGD_2 is appropriate in the supernatants of cell cultures where PGD_2 levels may reach several ng/ml, in purified enzyme preparations and in CSF, where concentrations of several hundred pg/ml have been measured.[6]

About This Assay

All studies of PGD_2 biosynthesis should take into consideration the chemical instability of PGD_2 and its rapid degradation in the presence of serum proteins such as albumin.[7] PGD_2 also readily degrades in both acidic and basic conditions to give a variety of decomposition products. Our PGD_2-MOX EIA Kit (Item No. 512011) is suited for the measurement of PGD_2 in a variety of complex sample matrices but requires conversion of PGD_2 to a stable methoxylamine derivative prior to performing the assay. Cayman's PGD_2 Fluorescence Polarization Immunoassay (FPIA) is especially designed for the rapid measurement of PGD_2 from cell culture and purified enzyme preparation without prior conversion to the methoxylamine compound. The PGD_2-FPIA is robust (Z' = 0.62), exhibits greater than 180 mP over a range of 340 pg/ml to 100 ng/ml PGD_2, and has a detection limit of 550 pg/ml.

Introduction to FPIA

Fluorescence polarization (FP) assays are homogeneous, single-step assays ideally suited for high-throughput screening (HTS) of large numbers of samples. All FP assays employ a large molecular species, or binding partner (BP) in conjunction with a small, low molecular weight fluorescent analyte (FA). When the large BP is an antibody, the assay is referred to as a fluorescence polarization immunoassay (FPIA).

Fluorescence is by definition the ability of a molecule to absorb the energy of an incoming (excitation) photon and then re-emit most of this energy as a new, slightly less energetic (emission) photon.

A small fluorescent molecule will rotate appreciably during the very small interval of time between absorption of a photon and emission of the fluorescence photon.

If the excitation light is polarized, this rotation will result in complete randomization of the plane of the emitted light. Thus, small fluorescent molecules depolarize an excitation pulse of polarized light (see Figure 1, below).

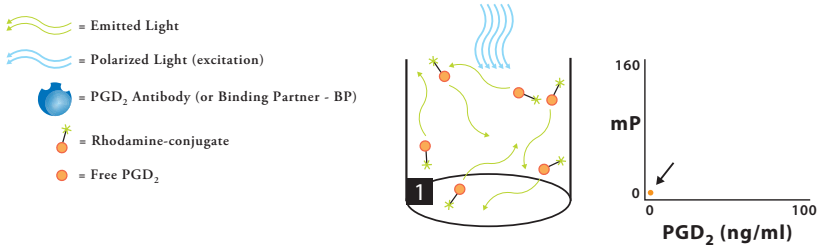


Figure 1.

Large fluorescent molecules (MW >100,000) do not rotate appreciably in the same small interval of time. They will therefore emit light that retains some of the polarization of the polarized excitation light. This polarization is quantified as milli-polarization units, or mP. A fluorescence polarization reader is required to make this measurement.

When a small fluorescent molecule becomes tightly bound to a large one, as in the binding of the PGD_2 rhodamine to PGD_2 antibody, the rotational speed of the small molecule is abruptly reduced to that of the entire complex (see Figure 2, below).

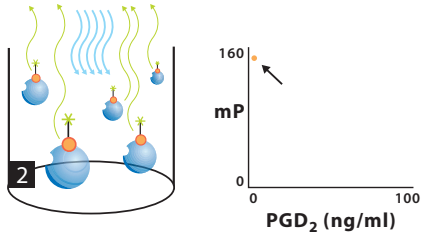


Figure 2.

Therefore, PGD_2-rhodamine bound to its antibody represents a large fluorescent molecule, which exhibits a high degree of FP. A microplate well filled with the PGD_2-rhodamine:antibody complex will give a high FP reading. The PGD_2 FPIA is based on the competition of free PGD_2 in the samples or standards for the high affinity binding site of a PGD_2 monoclonal antibody occupied by a rhodamine-conjugate. Addition of a small amount of natural, unlabeled PGD_2 will result in a competition between the unlabeled PGD_2 and the rhodamine-conjugate for the antibody (see Figure 3 below).

Figure 3.

Some of the fluorescent rhodamine-conjugate will be released from the antibody, and will resume its intrinsic, rapid rate of rotation. This will cause a detectable loss of FP in the well (see Figure 4, below).

The addition of large amount of PGD_2 (50-100 ng/ml) will result in a much larger reduction in the mP of the well (see Figure 5, on page 9). Plotting mP *versus* PGD_2 allows the construction of a standard curve with a broad dynamic range. This is similar to, but not strictly analagous to, the sigmoidal dose-response curve in a traditional solid phase EIA.

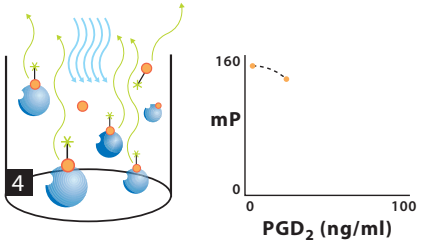


Figure 4.

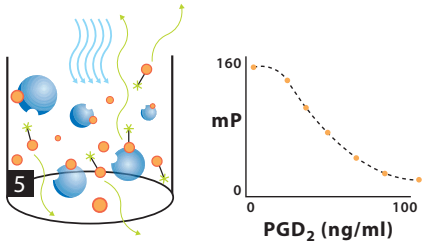


Figure 5.

Cayman's PGD_2 FPIA - Red gives accurate quantitation of PGD_2 in the range of 340 pg/ml - 100 ng/ml within 60 minutes. Any samples falling outside this range should be diluted or concentrated prior to analysis.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all FPIA reagents and buffers must be deionized and free of trace organic contaminants ("UltraPure"). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for FPIA. UltraPure water may be purchased from Cayman Chemical (Item No. 400000).

Buffer Preparation

Dilute the contents of one vial of FPIA Buffer Concentrate (10X) (Item No. 400501) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.* Store the buffer at 4°C; it will be stable for approximately one month.

Sample Preparation

a. All samples must be free of organic solvents prior to assay

b. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Cultured cells

Samples containing culture medium cannot be used directly in the assay with the following exceptions. Phenol red-free DMEM can be used in the assay directly without dilution. Phenol red-free DMEM with 10% FBS can be used in the assay at a minimum of 1:5 dilution. Variations of this methodology may be employed, provided care is taken to assess potential interference from any buffers utilized in the assay.

In vitro Enzyme Assays

The PGD_2 FPIA is well suited for analysis of PGD_2 concentrations from *in vitro* assay systems (*i.e.*, microsomal or purified preparations of COX and PGD synthase or analogous expression systems). The buffer utilized in the assay system should be checked for interference in the FPIA as described below (See **Testing for Interference in a Sample Matrix** on page 14).

ASSAY PROTOCOL

Preparation of Assay Specific Reagents

PGD_2 FPIA Reagent - Red

Reconstitute the PGD_2 FPIA Reagent - Red as follows:

400 dtn PGD_2 FPIA Reagent - Red (384-well kit; Item No. 10010445): Reconstitute with 12 ml FPIA Buffer.

OR

2,000 dtn PGD_2 FPIA Reagent - Red (1,920-well kit; Item No. 10010445): Reconstitute with 60 ml FPIA Buffer.

NOTE: 12 ml of reconstituted reagent is enough for either a standard 96- or 384-well plate. 60 ml of reconstituted reagent is enough for either five standard 96- or 384-well plates. Allow the reagent to equilibrate to room temperature prior to use. Store unused PGD_2 FPIA Reagent - Red at 4°C, *protect it from light*, and use within 48 hours.

PGD_2 FPIA Standard - Red

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the PGD_2 FPIA Standard - Red (Item No. 412054) into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 1 µg/ml. This solution can be stored at 4°C for up to eight hours.

To prepare the standard for use in the FPIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl FPIA Buffer to tube #1 and 500 µl FPIA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (1 µg/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. The diluted standards should be used immediately.

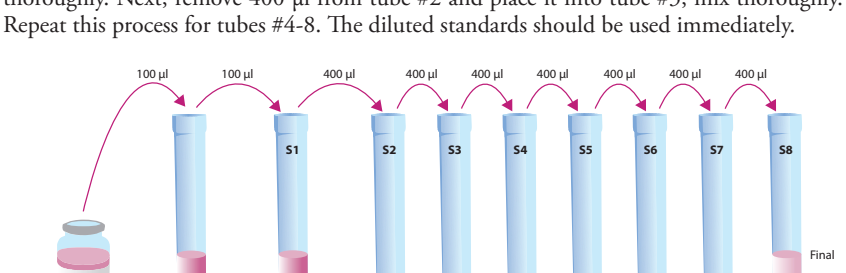


Figure 6. Preparation of the PGD_2 standards

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Do not expose the pipette tip to the reagent(s) already in the well.
- Avoid introducing bubbles into the wells.

Follow the steps below to accurately measure mP in the assay. Allow all reagents to equilibrate to room temperature prior to performing the assay. *NOTE: Volumes shown are for a 384-well plate format. For a 96-well plate format, use 100 µl of standard, sample, and FPIA Reagent.*

1. PGD_2 FPIA Standard - Red

a. Add 25 µl from tube #8 to both of the lowest standard wells (S8). Add 25 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

b. Add 25 µl of FPIA Buffer to a minimum of two wells. These wells will serve as the maximum mP (mP_{max}) wells and will quantify the maximum signal possible in the absence of any unlabeled PGD_2.

2. Samples

Add 25 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed at least in duplicate (triplicate recommended).

Optional Step

Read and store the emission for each well in both parallel and perpendicular modes (see details on reading the plate in step 5 below). These values can be subtracted from the corresponding values in the presence of the PGD_2 FPIA Reagent to eliminate any background fluorescence in the sample, thereby increasing the accuracy of the assay.

3. PGD_2 FPIA Reagent - Red

Add 25 µl of FPIA Reagent to each of the wells on the plate.

4. Incubation of the Plate

Cover the plate with the plate cover supplied in the kit and incubate for 60-90 minutes at room temperature on an orbital shaker. The FP signal is stable for at least three hours.

5. Reading the Plate

Read the plate(s) with excitation and emission wavelengths of 560 nm and 645 nm, respectively. The plate reader used at Cayman Chemical also employs a dichroic filter at 590 nm. Some instruments may not utilize this type of filter. The excitation polarizer is set in the horizontal position, and the emission polarizer is dynamic. The measurements are taken with the z-height set to the middle of the well and the G-factor is set to one.

Testing for Interference

The types of buffers and samples potentially utilized in this assay can vary widely. To test for interference in the sample matrix perform the following test. Prepare 2-fold serial dilutions of the sample matrix in the FPIA Buffer beginning at a 1:2 dilution (*i.e.*, make dilutions of 2, 4, 8, 16, 32, 64, etc.). Add reagents to the plate as described above and measure the mP_{max} in the presence of the undiluted matrix and for each of the dilutions. As a control, measure mP using only FPIA Buffer with the FPIA Reagent - Red.

If there is no interference from the sample matrix, the mP values at all dilutions will be at the mP_{max} level. Interference will cause a reduction in mP that will diminish as dilutions of the sample matrix increase. A minimum dilution required for removal of the interference can be easily determined using a plot of mP *versus* dilution. It may be possible to perform the assay at suboptimal sample matrix dilutions, provided the standard and all samples contain the same amount of sample matrix and therefore exhibit the same level of signal suppression.

ANALYSIS

Calculations

Fluorescence polarization of a molecule is defined as:

$$\text{Polarization (mP)} = 1{,}000 \times \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + I_{\perp})} \qquad \text{Range: 0 to 500 mP}$$

A plot of mP *versus* PGD_2 concentration on semi-log axes results in a sigmoidal dose-response curve typical of competitive binding assays. This data can be fit to a 4-parameter logistic equation as shown in Figure 7 (see page 18).

A second method of data analysis uses a logit-log plot. The logit-log method is a transformation based on the following equation:

$$\text{logit}(y) = \ln[y/(1-y)] \qquad \text{where } y = (mP_{\text{standard or sample}}/mP_{max})$$

The logit transformation reduces the sigmoidal curve of mP *versus* log concentration to a straight line of logit $mP_{standard}/mP_{max}$ *versus* PGD_2 on semi-log axes. The curve is completely described by the y-intercept and the slope of the line, which can be used to calculate the concentration values from the logit mP of the samples.

Performance Characteristics

Z'-Factor

Z'-factor is a term used to describe the quality of an assay,[8] which is calculated using the following equation:

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{| \mu_{c+} - \mu_{c-} |}$$

The theoretical upper limit for the Z′-factor is 1.0. A robust assay has a Z′-factor >0.5. The Z′-factor for Cayman's PGD_2 FPIA - Red was determined to be 0.62.

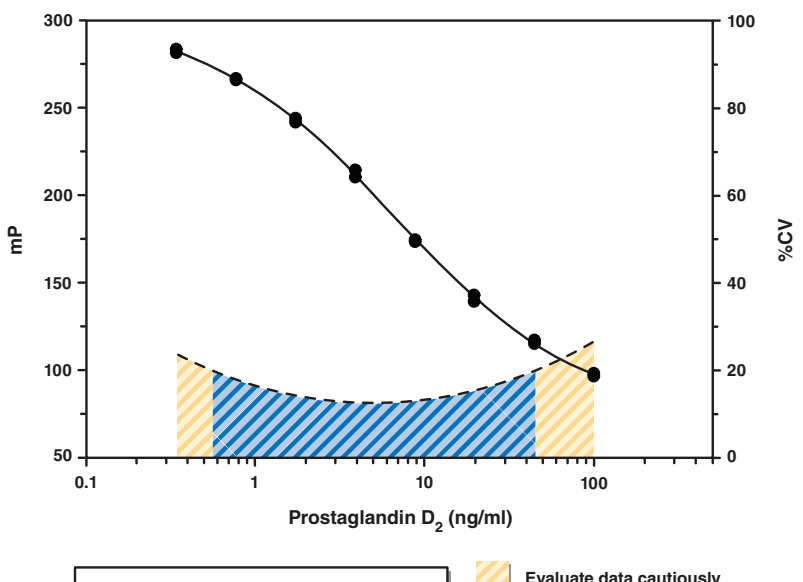
Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Dose (ng/ml)	mP		Average mP	%CV*
100	98	96	97	26.5
44.4	117	115	116	20.0
19.8	143	139	141	15.5
8.8	174	173	174	12.3
3.9	210	214	212	13.7
1.7	242	244	243	13.0
0.8	266	267	266	18.7
0.3	282	283	283	23.5

Table 3. Typical data for the PGD_2 standard curve

*%CV represents the variation in concentration (not absorbance) of 40 repetitions of each point on the standard curve as determined using a reference standard curve.



Average ΔmP = 185 mP
Detection Limit = 550 pg/ml

Figure 7. PGD_2 standard curve fit to a 4-parameter logistic equation

Specificity

Compound	Cross Reactivity
Prostaglandin $F_{2\alpha}$	118%
Prostaglandin D_2	100%
Prostaglandin J_2	24%
11β-Prostaglandin $F_{2\alpha}$	4.1%
12(S)-HHTrE	3.6%
Thromboxane B_2	3.5%
Prostaglandin E_2	2.5%
6-keto Prostaglandin $F_{1\alpha}$	0.46%
13,14-dihydro-15-keto Prostaglandin D_2	0.08%
Arachidonic Acid	0.06%

Table 2. Specificity

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
High background mP	A. High protein concentration in sample matrix B. Fluorescent molecules in sample matrix	A. Test sample matrix for interference before running samples in the assay

References

1. Urade, Y., Tanaka, T., Eguchi, N., *et al.* Structural and functional significance of cysteine residues of glutathione-independent prostaglandin D synthase. *J. Biol. Chem.* **270**, 1422-1428 (1995).
2. Satoh, S., Matsumura, H., Nakajima, T., *et al.* Promotion of sleep by prostaglandin D_2 in rats made insomniac by pretreatment with para-chlorophenylalanine. *Neurosci. Res.* **21**, 41-50 (1995).
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4. Roberts, L.J., II and Sweetman, B.J. Metabolic fate of endogenously synthesized prostaglandin D_2 in a human female with mastocytosis. *Prostaglandins* **30**, 383-400 (1985).
5. Song, W.-L., Wang, M., Ricciotti, E., *et al.* Tetranor PGDM, an abundant urinary metabolite reflects biosynthesis of prostaglandin D_2 in mice and humans. *J. Biol. Chem.* **283(2)**, 1179-1188 (2008).
6. Ujihara, M., Urade, Y., Eguchi, N., *et al.* Prostaglandin D_2 formation and characterization of its synthetases in various tissues of adult rats. *Arch. Biochem. Biophys.* **260**, 521-531 (1988).
7. Fitzpatrick, F.A. and Wynalda, M.A. Albumin-catalyzed metabolism of prostaglandin D_2. Identification of products formed *in vitro*. *J. Biol. Chem.* **258**, 11713-11718 (1983).
8. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *Journal of Biomolecular Screening* **4(2)**, 67-73 (1999).

Related Products

Prostaglandin D Synthase Inhibitor Screening Assay Kit - Item No. 10006595
Prostaglandin D_2 - Item No. 12010
PProstaglandin D_2 EIA Kit - Item No. 512031
Prostaglandin D_2 Express EIA Kit - Item No. 512041
Prostaglandin D_2 FPIA Kit - Green - Item No. 500581
rostaglandin D_2-MOX EIA Kit - Item No. 512011
Prostaglandin D_2-MOX Express EIA Kit - Item No. 500151
Prostaglandin E_2 FPIA Kit - Green - Item No. 500501
Prostaglandin E_2 FPIA Kit - Red - Item No. 10004517
UltraPure Water - Item No. 400000

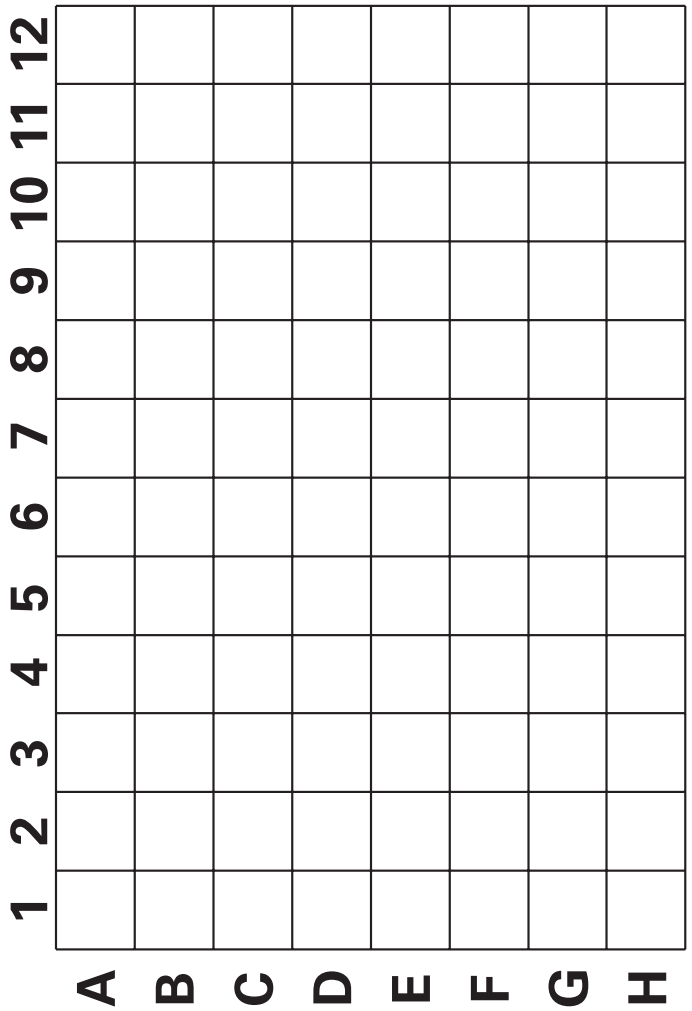
Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material **will meet our specifications at the time of delivery**. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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