Nampt/Visfatin (mouse/rat) EIA Kit

Item No. 579040

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Vial Number	Item	96 wells Quantity/Size	Storage
1	Anti-Nampt/Visfatin (mouse) Precoated 96-Well Strip Plate	1 plate	4°C
2	Wash Buffer Concentrate (10X)	2 vials/30 ml	4°C
3	Dilution Buffer (10X)	2 vials/30 ml	4°C
4	Detection Antibody	1 vial/50 µl	4°C
5	Anti-IgG/HRP Conjugate (100X)	1 vial/150 µl	4°C
6	Nampt/Visfatin (mouse) EIA Standard	1 vial	4°C
7	Substrate Solution	1 vial/12 ml	4°C
8	Stop Solution	1 vial/12 ml	4°C
9	Plate Cover	3 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 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 Cayman's Nampt/Visfatin (mouse/rat) EIA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

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 and used before the expiration date indicated on the outside of the box. Reagents must be stored at 4°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted Wash Solution (see page 9) may be stored at room temperature for up to one month.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled water or deionized water is acceptable.
4. Phosphate buffered saline.
5. Materials used for **Sample Preparation** (see page 10).

INTRODUCTION

Background

Nicotinamide phosphoribosyltransferase (Nampt) is the rate-limiting enzyme in the salvage pathway for the biosynthesis of nicotinamide adenine dinucleotide (NAD^+) from nicotinamide.[1] NAD^+ is an essential coenzyme involved in cellular metabolism. Nampt was first described as a pre-B-cell colony enhancing factor (PBEF).[2] It was later named visfatin, as it was found to be highly enriched in visceral fat, with plasma levels increasing with obesity.[3] This study was retracted because claims that visfatin activates the insulin receptor could not be verified.[4]

Whether it is called Nampt, PBEF, or Visfatin, this protein has important roles, both intra- and extracellularly, in cellular metabolism and disease. Within all cell types, Nampt's role in controlling NAD^+ synthesis impacts many cellular pathways as well as cell survival. In mesangial cells in the kidney, Nampt increases NAD^+ levels, which in turn increases glucose uptake, suggesting a role for Nampt in diabetic nephropathy.[5] Exercise increases Nampt levels in skeletal muscle, which appears to modulate energy metabolism in a NAD^+- and sirtuin-dependant manner.[6] Both intracellular Nampt and NAD^+ display circadian oscillations and are directly linked to rhythms in cellular metabolism.[7] Importantly, inhibition of Nampt activity selectively targets cancer cells for apoptosis because cancer cells have an unusually high rate of NAD^+ turnover.[1,8,9]

Extracellularly, the levels of Nampt in serum correlate with body mass index and body fat mass, are increased during inflammation, and are decreased with liver cirrhosis.[10-12] Extracellular Nampt regulates insulin secretion in β cells by regulating systemic NAD^+ biosynthesis.[13] Nampt levels and expression in serum, circulating leukocytes, and tissues may be useful biomarkers for inflammation, cancer, obesity, and other diseases.[1,14,15]

About This Assay

Cayman's Nampt/Visfatin (mouse/rat) EIA Kit is an immunometric assay which can be used to measure Nampt/Visfatin in mouse or rat serum. The assay exhibits a detection limit of 50 pg/ml and an assay range of 0-32 ng/ml.

Principle of the Assay

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for Nampt/Visfatin. This antibody will bind any Nampt/Visfatin introduced into the well. Standards and samples are incubated on the antibody-coated plate, and the plate is then washed before addition of a purified polyclonal anti-Nampt/Visfatin antibody to detect the captured Nampt/Visfatin. An anti-IgG/HRP-conjugate is used to recognize the 'sandwiches'. The concentration of the analyte is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound anti-IgG/HRP conjugate, which in turn is proportional to the concentration of the Nampt/Visfatin.

$$\textbf{Absorbance} \propto [\textbf{anti-IgG/HRP}] \propto [\textbf{visfatin}]$$

A schematic of this process is shown in Figure 1, on page 8.

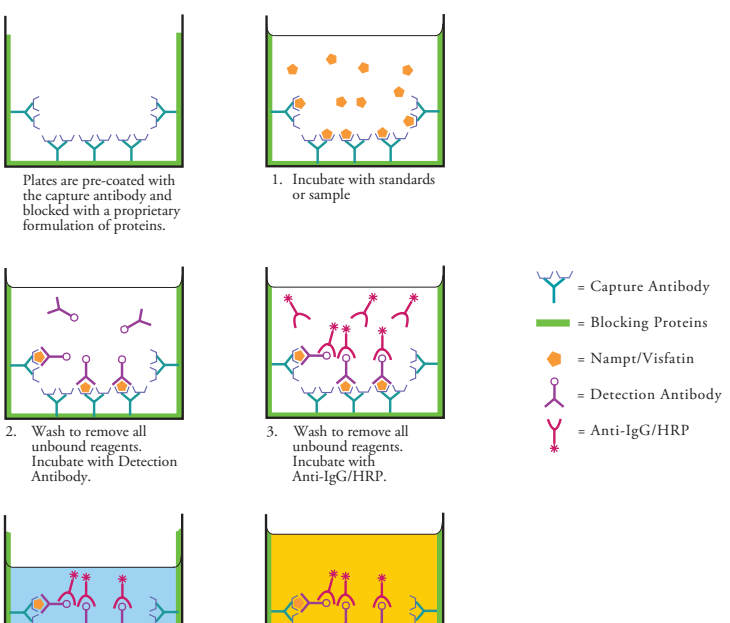

Figure 1. Schematic of the immunometric EIA

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months.

1. Dilution Buffer Preparation

To prepare a 1X Dilution Buffer Solution, dilute the Dilution Buffer (10X) (vial #3) 1:10 with deionized water (*i.e.*, mix 1 part Dilution Buffer (10X) with 9 parts deionized water).

2. Wash Buffer Preparation

To prepare a 1X Wash Buffer Solution, dilute the Wash Buffer Concentrate (10X) (vial #2) 1:10 with deionized water (*i.e.*, mix 1 part Wash Buffer Concentrate (10X) with 9 parts deionized water).

3. Detection Antibody Preparation

Dilute the Detection Antibody (vial #4) 1:250 with 1X Dilution Buffer (*i.e.*, mix 1 part Detection Antibody with 249 parts 1X Dilution Buffer). The diluted Detection Antibody is not stable and cannot be stored.

4. Anti-IgG/HRP Conjugate Preparation

Dilute the Anti-IgG/HRP Conjugate (100X) (vial #5) 1:100 with 1X Dilution Buffer (*i.e.*, mix 1 part Anti-IgG/HRP Conjugate (100X) with 99 parts 1X Dilution Buffer). Use the 1X Anti-IgG/HRP Conjugate within one hour of preparation.

5. Substrate Solution Preparation

Prior to use, warm the Substrate Solution (vial #7) to room temperature. The solution is ready to use as supplied.

Sample Preparation

Serum

Allow blood samples to clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000 x g. Assay freshly prepared serum or store serum in aliquots at ≤-20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma

Assaying plasma samples is not recommended.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Nampt/Visfatin (mouse) EIA Standard

When opening the lyophilized standard, remove the cap gently as the powder may be loose within the vial. Reconstitute the lyophilized purified Nampt/Visfatin (mouse) EIA Standard (vial #6) with 1 ml of deionized water. Mix gently. The concentration of this solution (the bulk standard) is 64 ng/ml. We recommend that unused Nampt/Visfatin EIA Standard be aliquotted and stored at -20°C.

To prepare the standard for use in EIA: Obtain eight clean microcentrifuge tubes and label them #1 through #8. Aliquot 300 µl of Dilution Buffer to tubes #1-8. Transfer 300 µl of freshly prepared bulk standard (64 ng/ml) to tube #8. Mix gently. The concentration of this standard is 32 ng/ml. Dilute the standard by removing 300 µl from tube #8 and placing into tube #7. Mix gently. Next, remove 300 µl from tube #7 and place into tube #6; mix gently. Repeat this process for tubes #5 to #2. Do not add any Nampt/Visfatin to tube #1. This tube is the zero-point vial, the lowest point on the standard curve and will serve as the blank.

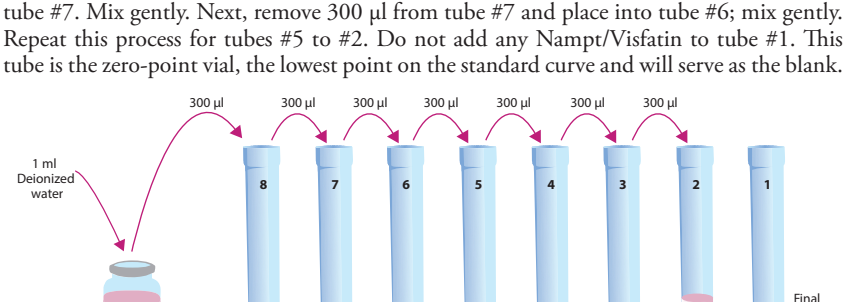

Figure 2. Preparation of the Nampt/Visfatin (mouse) standards

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, place the unused strips back in the plate packet and store according to the plate insert at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 3, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 15, for more details). We suggest you record the contents of each well on the template sheet provided (see page 27).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	9	9	17	17	25	25	33	33
B	S2	S2	2	2	10	10	18	18	26	26	34	34
C	S3	S3	3	3	11	11	19	19	27	27	35	35
D	S4	S4	4	4	12	12	20	20	28	28	36	36
E	S5	S5	5	5	13	13	21	21	29	29	37	37
F	S6	S6	6	6	14	14	22	22	30	30	38	38
G	S7	S7	7	7	15	15	23	23	31	31	39	39
H	S8	S8	8	8	16	16	24	24	32	32	40	40

S1-S8 - Standards 1-8
1-40 - Samples

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- 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.

Addition of Standards and Samples and First Incubation

1. Add 100 µl of the standards or diluted sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with plate cover and incubate overnight at 4°C.

Addition of Detection Antibody and Second Incubation

1. Empty the wells and rinse three times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of the Detection Antibody to each well of the plate.
3. Cover the plate with plate cover and incubate for one hour at 37°C.

Addition of Anti-IgG/HRP Conjugate and Third Incubation

1. Empty the wells and rinse three times with Wash Buffer.
2. Add 100 µl of Anti-IgG/HRP Conjugate to each well of the plate.
3. Cover the plate with plate cover and incubate for one hour at 37°C.

Development of the Plate

1. Empty the wells and rinse five times with Wash Buffer.
2. Add 100 µl of Substrate Solution to each well of the plate.
3. Cover the plate with plate cover and incubate for 10 minutes at room temperature in the dark.
4. DO NOT WASH THE PLATE. Add 100 µl of Stop Solution (vial #8) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. We recommend using a second order polynomial (quadratic) fit since the absorbance at the standard concentrations is slightly non-linear. *NOTE: Cayman has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

1. Average the absorbance values for wells of the zero-point standard.
2. Subtract the average absorbance reading of the zero point standard from the absorbance of all wells of the plate.

Plot the Standard Curve

Plot absorbance *versus* concentration for standards (S1-S8) using linear x- and y-axis and fit the data with a quadratic equation. (Option: Plot concentration of the standard on the y-axis *versus* the absorbance on the x-axis.)

Determine the Sample Concentration

1. Use a calculator or spreadsheet to determine the quadratic regression line.
2. Use the equation of the curve generated by the regression fit to calculate the value of your samples.

Performance Characteristics

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.

Nampt/Visfatin (ng/ml)	Raw Data	Blank Subtracted
0	0.162	0.000
0.5	0.194	0.032
1	0.224	0.063
2	0.294	0.132
4	0.454	0.292
8	0.756	0.594
16	1.256	1.094
32	2.092	1.930

Table 1. Typical results

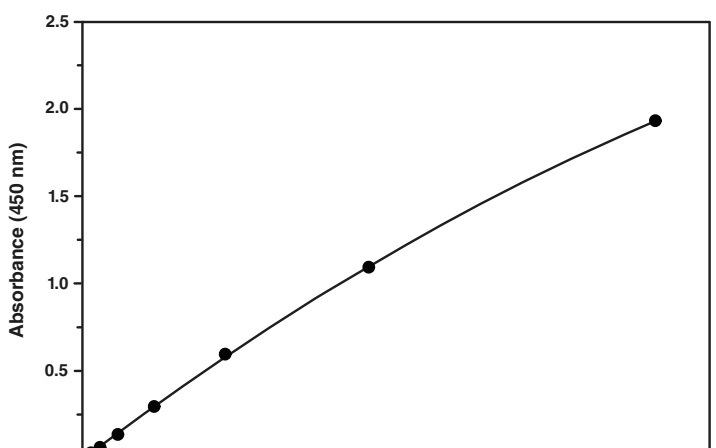

Figure 4. Typical standard curve
The minimum detectable concentration is 50 pg/ml.

Precision:

a. Intra-Assay Precision

Six samples were tested five times to assess the intra-assay precision.

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	%CV
1	5.6	0.30	5.30
2	16.7	1.04	6.21
3	9.65	0.43	4.44
4	13.6	0.06	0.41
5	10.6	0.17	1.64
6	19.8	0.36	1.84

Table 2. Intra-assay variation

b. Inter-Assay Precision

Six samples were tested six times to assess the inter-assay precision.

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	%CV
1	8.20	0.72	8.80
2	7.93	0.75	9.44
3	12.6	0.84	6.68
4	10.8	0.29	2.69
5	19.4	0.91	4.71
6	8.99	0.44	4.87

Table 3. Inter-assay variation

Specificity:

Analyte (recombinant proteins)	Max Concentration (ng/ml)	Cross Reactivity (%)
Mouse Nampt/Visfatin	10	100
Rat Nampt/Visfatin	10	100
Mouse Adiponectin	100	N.R.
Mouse ANGPTL3	100	N.R.
Mouse ANGPTL4	100	N.R.
Mouse Clusterin	100	N.R.
Mouse GPX3	100	N.R.
Mouse IL-33	100	N.R.
Mouse Leptin	100	N.R.
Mouse Progranulin	100	N.R.
Mouse RBP4	100	N.R.
Mouse Resistin	100	N.R.
Mouse TNF-α	100	N.R.
Mouse Vaspin	100	N.R.
Human Adiponectin	100	N.R.
Human AGF	100	N.R.
Human Nampt/Visfatin	100	N.R.

Table 4. Specificity of the Nampt/Visfatin (mouse) EIA Kit
N.R. = No Cross Reactivity

Recovery:

The recovery of Nampt/Visfatin spiked to three different levels in four different samples throughout the range of assay was evaluated.

Sample	Average Recovery (%)	Range (%)
1	100.7	95-105
2	99.5	90-100
3	100.7	95-105
4	104.7	95-105

Table 5. Recovery of Nampt/Visfatin (mouse) in serum

Linearity - Effect of serum dilution:

To assess the linearity of the assay, four serum samples were first diluted as indicated below prior to sample preparation as described in the protocol.

Sample	Serum Dilution	Expected (ng/ml)	Observed (ng/ml)	% of Expected
Mouse serum 1	1	6.02	6.02	100
	1/2	3.01	3.07	102
	1/4	1.50	1.40	93.3
Mouse serum 2	1	4.57	4.57	100
	1/2	2.29	1.90	83.2
	1/4	1.14	1.23	107.9
Rat serum 1	1	6.53	6.53	100
	1/2	3.27	3.38	103.3
	1/4	1.63	1.52	92.9
Rat serum 2	1	9.64	9.64	100
	1/2	4.82	4.60	95.4
	1/4	2.41	2.34	97.1

Table 6. Effect of serum dilution (% of expected = observed/expected x 100%)

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal	A. Omission of key reagent B. Washes too stringent C. Incubation times inadequate D. Plate reader settings not optimal E. Incorrect assay temperature	A. Check that all reagents have been added in the correct order B. Use an automated plate washer if possible C. Use recommended incubation times D. Verify the wavelength and/or filter settings in the plate reader E. Use recommended incubation temperature; bring substrates to room temperature before use
High background	A. Concentration of Anti-IgG/HRP Conjugate too high B. Inadequate washing	A. Use recommended dilution B. Ensure all wells are filled with Wash Buffer and are aspirated completely
Poor standard curve	A. Wells not completely aspirated B. Reagents poorly mixed C. Dilution error D. Technique problem	A. Completely aspirate wells between steps B. Be sure that reagents are thoroughly mixed C. Check pipetting technique and double-check calculations D. Proper mixing of reagents and wash steps are critical

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Related Products

Adipogenesis Assay Kit - Item No. 10006908
Adipolysis Assay Kit - Item No. 10009381
Adiponectin (human) EIA Kit - Item No. 500641
Adiponectin (human) EIA Kit (HS) - Item No. 10007619
Adiponectin (mouse) EIA Kit - Item No. 10007620
ANGPTL3 (human) EIA Kit - Item No. 580170
ANGPTL6 (human) EIA Kit - Item No. 580190
CAY10618 - Item No. 13670
Clusterin (human) EIA Kit - Item No. 580110
CTRP3 (human) EIA Kit - Item No. 580200
CTRP5 (human) EIA Kit - Item No. 580120
FABP4 (human) EIA Kit - Item No. 10007614
Ghrelin (human acylated) EIA Kit - Item No. 10006306
Ghrelin (human unacylated) EIA Kit - Item No. 10008952
Ghrelin (rat acylated) EIA Kit - Item No. 10006307
Ghrelin (rat unacylated) EIA Kit - Item No. 10008953
Leptin (human) EIA Kit - Item No. 500010
Leptin (mouse/rat) EIA Kit - Item No. 10007609
Leptin Receptor (human) EIA Kit - Item No. 10007608
Nampt/Visfatin (human) EIA Kit - Item No. 579020
β-Nicotinamide Mononucleotide - Item No. 16411
Progranulin (human) EIA Kit - Item No. 500940
Progranulin (mouse) EIA Kit - Item No. 500950
Resistin (human) EIA Kit - Item No. 10007610
Resistin (rat) EIA Kit - Item No. 10007612
Retinol Binding Protein 4 (human) Competitive EIA Kit - Item No. 579070
Vaspin (human) EIA Kit - Item No. 579000

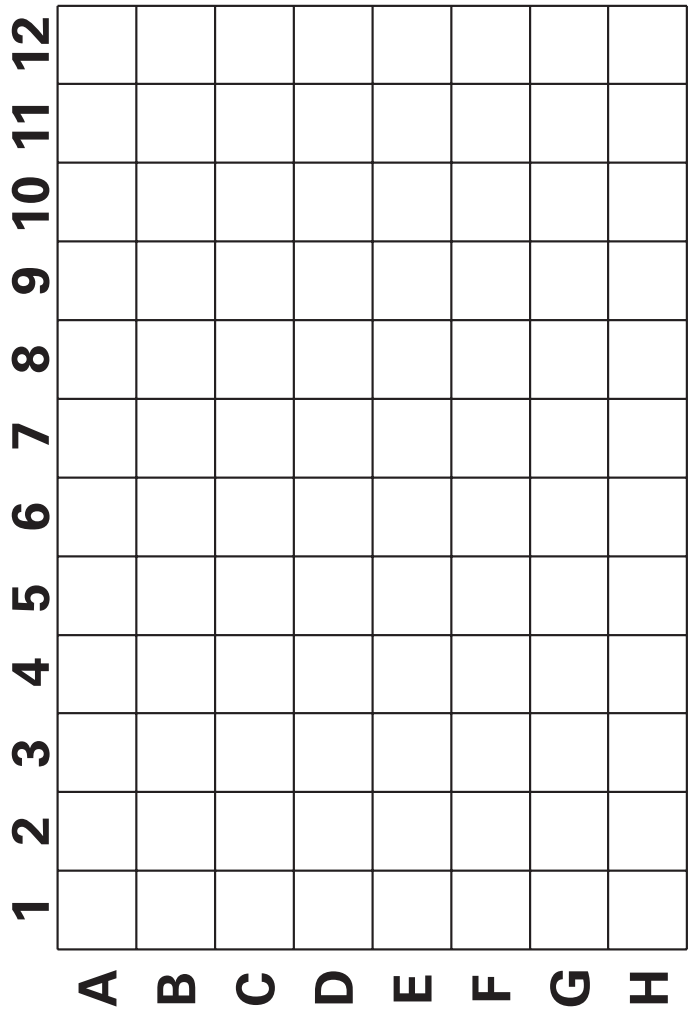
Warranty and Limitation of Remedy

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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