

Catalase Assay Kit

Item No. 707002



Customer Service 800.364.9897 * **Technical Support** 888.526.5351

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TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Precautions
	5	If You Have Problems
	5	Storage and Stability
	5	Materials Needed but Not Supplied
INTRODUCTION	6	Background
	7	About This Assay
PRE-ASSAY PREPARATION	8	Reagent Preparation
	10	Sample Preparation
ASSAY PROTOCOL	12	Plate Set Up
	14	Standard Preparation
	15	Performing the Assay
ANALYSIS	16	Calculations
	17	Performance Characteristics
	18	Linearity of the Assay
RESOURCES	19	Interferences
	20	Troubleshooting
	21	References
	21	Related Products
	22	Warranty and Limitation of Remedy
	23	Plate Template
	24	Notes

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
707010	Catalase Assay Buffer (10X)	1 vial/5 ml	2 vials/5 ml
707012	Catalase Sample Buffer (10X)	1 vial/10 ml	2 vials/10 ml
707014	Catalase Formaldehyde Standard	1 vial/100 µl	1 vial/100 µl
707013	Catalase (Control)	1 vial/lyophilized	2 vials/lyophilized
707015	Catalase Potassium Hydroxide	1 vial/4 ml	5 vials/4 ml
707011	Catalase Hydrogen Peroxide	1 vial/1 ml	1 vial/1 ml
707017	Catalase Purpald (Chromogen)	1 vial/4 ml	5 vials/4 ml
707018	Catalase Potassium Periodate	1 vial/1.5 ml	5 vials/1.5 ml
400010	High-Binding 96-Well Solid Plate	1 plate	5 plates
400012	96-Well Cover Sheet	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) 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.

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

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

Catalase Hydrogen Peroxide is corrosive and is 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. Keep away from combustible materials.*

Catalase Formaldehyde Standard is carcinogenic. It is toxic if inhaled, ingested, or if in contact with skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.*

Catalase Potassium Hydroxide is corrosive and is 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. Keep away from combustible materials.*

Catalase Purpald (Chromogen) is an irritant. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.*

Catalase Potassium Periodate is an oxidizer and an irritant. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.*

Hydrochloric acid is corrosive and is 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.*

***Before use the user must review the complete Material Safety Data Sheet.**

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 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 with a 540 nm filter.
2. An adjustable pipettor and a repeating pipettor.
3. A source of pure water. Glass distilled water or HPLC-grade water is acceptable.
4. A 5 ml vial of methanol can be purchased from Cayman (Item No. 707016).

INTRODUCTION

Background

Catalase (EC 1.11.1.6; 2H₂O₂ oxidoreductase) is an ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H₂O₂), a reactive oxygen species (ROS), which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. This enzyme catalyzes the conversion of two molecules of H₂O₂ to molecular oxygen and two molecules of water (catalytic activity). CAT also demonstrates peroxidatic activity, in which low molecular weight alcohols can serve as electron donors. While aliphatic alcohols serve as specific substrates for CAT, other enzymes with peroxidatic activity do not utilize these substrates.



In humans, the highest levels of CAT are found in liver, kidney, and erythrocytes, where it is believed to account for the majority of H₂O₂ decomposition.

About This Assay

Cayman's Catalase Assay Kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H₂O₂. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen.^{1,2} Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to a purple color.^{1,2} The assay can be used to measure CAT activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates.

PRE-ASSAY PREPARATION

Reagent Preparation

NOTE: Methanol is no longer supplied in this kit. It can be purchased separately under Item No. 707016 or you can supply your own.

1. Catalase Assay Buffer (10X) – (Item No. 707010)

Each vial contains 5 ml of Assay Buffer. Dilute 2 ml of Catalase Assay Buffer concentrate with 18 ml of HPLC-grade water. This final Assay Buffer (100 mM potassium phosphate, pH 7.0) should be used in the assay. When stored at 4°C, this diluted Assay Buffer is stable for at least two months. Prepare the additional vial as needed.

2. Catalase Sample Buffer (10X) – (Item No. 707012)

Each vial contains 10 ml of Sample Buffer. Dilute 5 ml of Catalase Sample Buffer concentrate with 45 ml of HPLC-grade water. This final Sample Buffer (25 mM potassium phosphate, pH 7.5, containing 1 mM EDTA and 0.1% BSA) should be used to dilute the formaldehyde standards, Catalase (Control), and CAT samples prior to assaying. When stored at 4°C, this diluted Sample Buffer is stable for at least two months. Prepare the additional vial as needed.

3. Catalase Formaldehyde Standard - (Item No. 707014)

The vial contains 4.25 M formaldehyde. The reagent is ready to use as supplied.

4. Catalase (Control) – (Item No. 707013)

Each vial contains a lyophilized powder of bovine liver CAT and is used as a positive control. Reconstitute the Catalase (Control) by adding 2 ml of diluted Sample Buffer to the vial and vortex well. Take 100 µl of the reconstituted enzyme and dilute with 1.9 ml of diluted Sample Buffer. A 20 µl aliquot of this diluted enzyme per well causes an absorbance of approximately 0.29 after subtracting the background absorbance. The diluted enzyme is stable for 30 minutes. The reconstituted Catalase (Control) is stable for one month at -20°C.

5. Catalase Potassium Hydroxide – (Item No. 707015)

Each vial contains 4 ml of 10 M potassium hydroxide (KOH). The reagent is ready to use as supplied.

6. Catalase Hydrogen Peroxide – (Item No. 707011)

The vial contains an 8.82 M solution of H₂O₂. Dilute 40 µl of Catalase Hydrogen Peroxide with 9.96 ml of HPLC-grade water. The diluted Hydrogen Peroxide solution is stable for two hours.

7. Catalase Purpald (Chromogen) – (Item No. 707017)

Each vial contains 4 ml of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) in 0.5 M hydrochloric acid. The reagent is ready to use as supplied.

8. Catalase Potassium Periodate – (Item No. 707018)

Each vial contains 1.5 ml of potassium periodate in 0.5 M potassium hydroxide. The reagent is ready to use as supplied.

Sample Preparation

Overheating can inactivate catalase. The enzyme should be kept cold during sample preparation and assaying. In general, catalase is very unstable at high dilution. It is recommended to store samples concentrated and assay within 30 minutes after dilution.

Tissue Homogenate

1. Prior to dissection, either perfuse tissue or rinse tissue with a phosphate buffered saline (PBS) solution, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue on ice in 5-10 ml of cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA) per gram tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Cell Lysate

1. Collect cells by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Homogenize or sonicate the cell pellet on ice in 1-2 ml of cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Plasma and Erythrocyte Lysate

1. Collect blood using an anticoagulant such as heparin, citrate, or EDTA.
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 until assaying or freeze at -80°C. The plasma sample will be stable for at least one month.
3. Remove the white buffy layer (leukocytes) and discard.
4. Lyse the erythrocytes (red blood cells) in four times its volume of ice-cold HPLC-grade water.
5. Centrifuge at 10,000 x g for 15 minutes at 4°C.
6. Collect the supernatant (erythrocyte lysate) for assaying and store on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for at least one month.

Serum

1. Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at 25°C.
2. 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 sample will be stable for at least one month.

Tissue Homogenization using the Precellys 24 Homogenizer

- Prior to dissection, either perfuse or rinse tissue with phosphate buffered saline (PBS), pH 7.4, to remove any red blood cells and clots.
- Freeze organs immediately upon collection and then store at -80°C. Snap-freezing of tissues in liquid nitrogen is preferred.
- Add cold 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA.
- Homogenize the tissue sample using the Precellys 24 according to appropriate settings.
- Spin the tissue homogenates at 10,000 x g for 15 minutes at 4°C.
- Collect supernatant and assay samples according to the kit booklet protocol. Samples may need to be diluted appropriately for assay and should be normalized using a protein assay.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that there be at least two wells designated as positive controls. A typical layout of formaldehyde standards and samples to be measured in duplicate is shown in Figure 1. We suggest you record the contents of each well on the template sheet provided on page 23.

	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	(+)	(+)	(S8)	(S8)	(S16)	(S16)	(S24)	(S24)	(S32)	(S32)	(S40)	(S40)

A-G = Standards

+ = Positive Controls

S1-S40 = Sample Wells

Figure 1. 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

- The final volume of the assay is 240 μ l in all the wells.
- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- If the expected CAT activity of the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the samples and formaldehyde standards be assayed at least in duplicate.
- Use the diluted Assay Buffer in the assay.
- Monitor the absorbance at 540 nm using a plate reader.

Standard Preparation

1. Preparation of the Formaldehyde Standards - Dilute 10 µl of Catalase Formaldehyde Standard (Item No. 707014) with 9.99 ml of diluted Sample Buffer to obtain a 4.25 mM formaldehyde stock solution. Take seven clean glass test tubes and mark them A-G. Add the amount of formaldehyde stock and diluted Sample Buffer to each tube as described in Table 1 (below).

Tube	Formaldehyde (µl)	Sample Buffer (µl)	Final Concentration (µM formaldehyde)*
A	0	1,000	0
B	10	990	5
C	30	970	15
D	60	940	30
E	90	910	45
F	120	880	60
G	150	850	75

Table 1

*Final formaldehyde concentration in the 170 µl reaction.

Performing the Assay

1. **Formaldehyde Standard Wells** - Add 100 µl of diluted Assay Buffer, 30 µl of methanol, and 20 µl of standard (tubes A-G) per well in the designated wells on the plate (see sample plate format, Figure 1, page 12).
2. **Positive Control Wells (bovine liver CAT)** - Add 100 µl of diluted Assay Buffer, 30 µl of methanol, and 20 µl of diluted Catalase (Control) to two wells.
3. **Sample Wells** - Add 100 µl of diluted Assay Buffer, 30 µl of methanol, and 20 µl of sample to two wells. To obtain reproducible results, the amount of CAT added to the well should result in an activity between 2-35 nmol/min/ml. When necessary, samples should be diluted with diluted Sample Buffer or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 100,000 to bring the enzymatic activity to this level.
4. Initiate the reactions by adding 20 µl of diluted Hydrogen Peroxide to all the wells being used. Make sure to note the precise time the reaction is initiated and add the diluted Hydrogen Peroxide as quickly as possible.
5. Cover the plate with the plate cover and incubate on a shaker for 20 minutes at room temperature.
6. Add 30 µl of Potassium Hydroxide to each well to terminate the reaction and then add 30 µl of Catalase Purpald (Chromogen) (Item No. 707017) to each well.
7. Cover the plate with the plate cover and incubate for 10 minutes at room temperature on the shaker.
8. Add 10 µl of Catalase Potassium Periodate (Item No. 707018) to each well. Cover with plate cover and incubate five minutes at room temperature on a shaker.
9. Read the absorbance at 540 nm using a plate reader.

Calculations

Determination of the Reaction Rate

1. Calculate the average absorbances of each standard and sample.
2. Subtract the average absorbance of standard A from itself and all other standards and samples.
3. Plot the corrected absorbance of standards (from step 2 above) as a function of final formaldehyde concentration (μM) from Table 1. See Figure 2 for a typical standard curve.

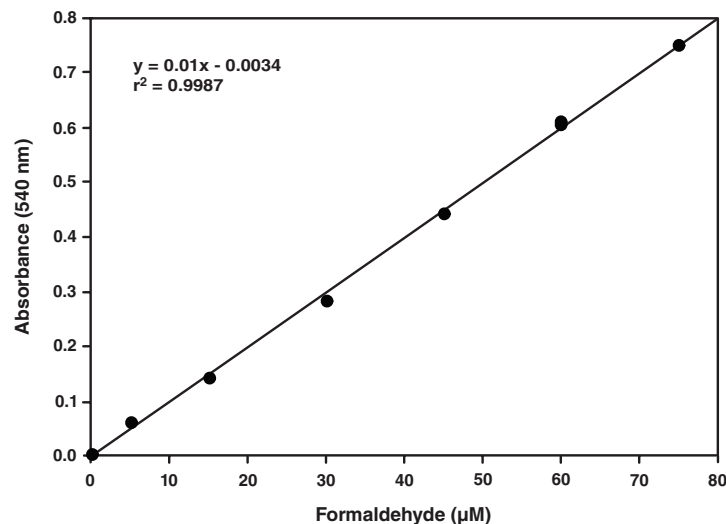


Figure 2. Formaldehyde standard curve

4. Calculate the formaldehyde concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample.

$$\text{Formaldehyde } (\mu\text{M}) = \left[\frac{\text{sample absorbance} - (\text{y-intercept})}{\text{slope}} \right] \times \frac{0.17 \text{ ml}}{0.02 \text{ ml}}$$

5. Calculate the CAT activity of the sample using the following equation. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C.

$$\text{CAT Activity} = \frac{\mu\text{M of sample}}{20 \text{ min.}} \times \text{Sample dilution} = \text{nmol/min/ml}$$

Performance Characteristics

Sensitivity:

The dynamic range of the assay is limited only by the accuracy of the absorbance measurement. Most plate readers are linear to an absorbance of 1.2. Samples containing CAT activity between 2-35 nmol/min/ml can be assayed without further dilution or concentration.

Precision:

When a series of 45 CAT measurements were performed on the same day, the intra-assay coefficient of variation was 3.8%. When a series of 45 CAT measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 9.9%.

Linearity of the Assay

The dose-response relationship for purified CAT from bovine liver was linear from 5-80 ng of protein (see Figure 3, below). Tissue homogenates, cell lysates, plasma, serum, and erythrocyte lysates also exhibited a linear relationship between the amount of sample and CAT activity over a wide range.

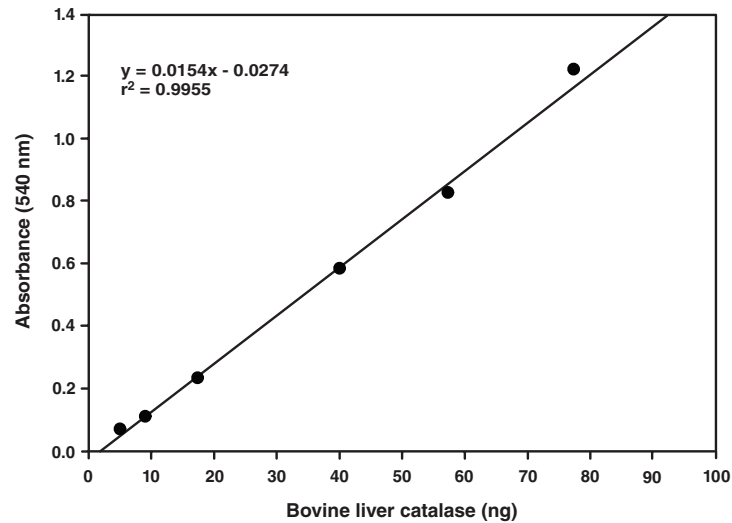


Figure 3. Absorbance *versus* bovine liver catalase (ng)

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

	Reagent	Will Interfere (Yes or No)
Detergents:	SDS (precipitates at pH 7.0)	Yes
	Triton X-100 (≤1%)	No
	Polysorbate 20 (≤1%)	No
	CHAPS (≤1%)	No
Buffers:	Tris	No
	HEPES	No
	Phosphate	No
Protease Inhibitors/Chelators:	Antipain (≤0.1 mg/ml)	No
	PMSF (≤1 mM)	No
	Leupeptin (≤1 mg/ml)	No
	Trypsin (≤0.1 mg/ml)	No
	Chymostatin (≤1 mg/ml)	No
	EGTA (≤1 mM)	No
	EDTA (≤1 mM)	No
Others:	NADPH (≤2 μM)	No
	Glycerol (≤1%)	No
	BSA (≤1 mg/ml)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	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 with your finger to remove bubbles
No activity was detected in the sample	A. Catalase activity was too low B. Sample was too dilute	Concentrate the samples using an Amicon concentrator with a molecular weight cut-off of 100,000 and re-assay
Absorbance over 1.2 in the sample wells	Too much enzyme was added to well(s)	Dilute samples with diluted Sample Buffer and re-assay
Absorbance of standard A is >0.2	The methanol is contaminated	Re-assay using methanol from a fresh container

References

1. Johansson, L.H. and Borg, L.A.H. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal. Biochem.* **174**, 331-336 (1988).

2. Wheeler, C.R., Salzman, J.A., Elsayed, N.M., *et al.* Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal. Biochem.* **184**, 193-199 (1990).

Related Products

Aconitase Assay Kit - Item No. 705502
Aconitase Fluorometric Assay Kit - Item No. 700600
Antioxidant Assay Kit - Item No. 709001
Ascorbate Assay Kit - Item No. 700420
Catalase Assay Kit (without Hydrogen Peroxide) - Item No. 700910
DNA/RNA Oxidative Damage EIA Kit - Item No. 589320
Glutathione Assay Kit - Item No. 703002
Glutathione Peroxide Assay Kit - Item No. 703102
Glutathione Reductase Assay Kit - Item No. 703202
Glutathione S-Transferase Assay Kit - Item No. 703302
Hydrogen Peroxide (urinary) Assay Kit - Item No. 706011
8-Isoprostane EIA Kit - Item No. 516351
Lipid Hydroperoxide (LPO) Assay Kit - Item No. 705002
Myeloperoxidase Chlorination Assay Kit - Item No. 10006438
Myeloperoxidase Inhibitor Screening Assay Kit - Item No. 700170
Myeloperoxidase Peroxidation Fluorometric Assay Kit - Item No. 700160
Neutrophil Myeloperoxidase Activity Assay Kit - Item No. 600620
Protein Carbonyl Colorimetric Assay Kit - Item No. 10005020
Protein Carbonyl Fluorometric Assay Kit - Item No. 700490
Sulfenylated Protein Cell-Based Detection Kit - Item No. 600320
Superoxide Dismutase Assay Kit - Item No. 706002
TBARS Assay Kit - Item No. 10009055
TBARS (TCA Method) Assay Kit - Item No. 700870
Thioredoxin Reductase Colorimetric Assay Kit - Item No. 10007892
Xanthine Oxidase Fluorometric Assay Kit - Item No. 10010895
For a complete list of related products please visit: www.caymanchem.com/catalog/707002

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.

12								
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	A	B	C	D	E	F	G	H

NOTES

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