

ION NaTRIUM Green-2 AM

2011C | 2011F

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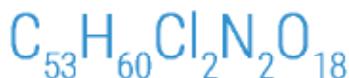
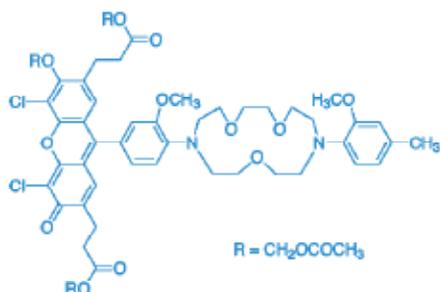
ION NaTRIUM Green-2 AM

CAT (VOL): 2011C (500 µg) | 2011F (10x50 µg)



Product Specifications

Membrane permeable acetoxymethyl (AM) ester derivative of ION NaTRIUM Green-2, a non-ratiometric green emission sodium indicator.



Molecular Weight	1084 g/mo
Solubility	DMSO
K_d	20mM ^a
Odor	None
Fire and Explosion Hazards	None
Shelf Life	Valid for one year after delivery, if stored properly (not dissolved in DMSO)

^a The dissociation constant (K_d) is sensitive to pH, temperature, viscosity, ionic strength, competing ions, and cellular interactions. These K_d's were measured in simple aqueous buffers as a guideline to the scientist, who should then calibrate the dye to his/her system.

TLC

Solvent	5% methanol in chloroform
R_f	0.5

HPLC

Column	C ₁₈
Detector Settings	254 nm, 460 nm
Purity	> 90%

Absorbance Spectrum

Solvent	Methanol
Absorbance max	469 ± 3 nm
ε	18,000 M ⁻¹ cm ⁻¹

Fluorescence Spectrum

Solvent	Methanol
Emission max	545 ± 3 nm
Excitation max	525 ± 3 nm

¹H NMR

All relevant peaks present	
Solvent	Deuterated acetone

Background

There is a large difference in the sodium ion (Na^+) concentration inside and outside the cell (5-40 mM intracellular; 120-450 mM extracellular, depending on organism). This concentration gradient is used to power nutrient uptake, to regulate concentrations of other intracellular ions and solutes, and to generate and transmit electrical impulses in excitable cells such as nerve and muscle. These functions are so important that organisms devote a major part of their metabolic energy to maintaining the sodium gradient. The low intracellular Na^+ concentration requires that a Na^+ indicator have the sensitivity to measure any small changes that occur. Moreover, intracellular potassium ion (K^+) concentration is typically high (in excess of 100 mM); therefore a Na^+ indicator should respond selectively to Na^+ , not K^+ .

ING-2 is a higher affinity analog of ING-1, as demonstrated by comparing the fluorimetric emission sodium chloride titrations of ING-1 (Figure 1) and ING-2 (Figure 2), with identical excitation and emission wavelengths. It retains the desirable properties of easy loading, slow leakage, and good photostability.

Figure 1

ION Natrium Green-1 Emission Spectra

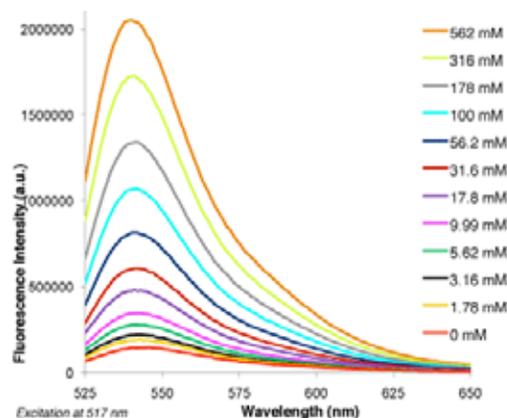
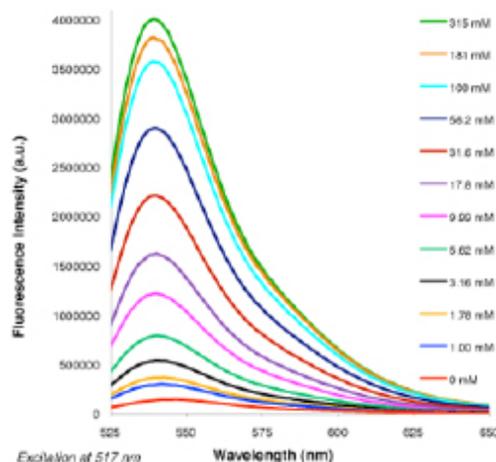


Figure 2

ION Natrium Green-2 Emission Spectra



Experimental Methods

Figure 3 Methods →

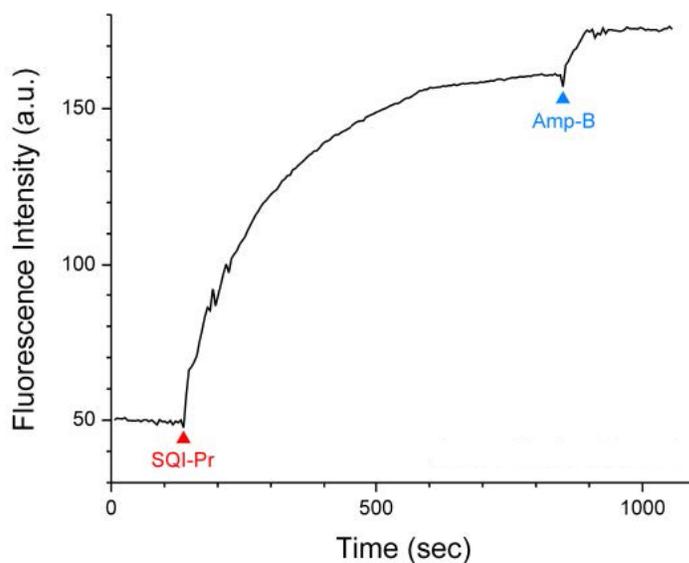
REF52 fibroblasts were incubated at room temperature for 60 minutes with 2 µM ION Natrium Green-2 AM ester in bicarbonate-buffered Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum, under an atmosphere of 5% CO₂/95% O₂; the medium also contained 0.0075% (w/v) of the nonionic surfactant, Pluronic F-127. The cells were then transferred into HBSS. SQI-Pr was prepared as a 20 mM stock solution in DMSO; amphotericin B was prepared as a 50 mM solution in DMSO. Appropriate volumes of the stock solutions were added to the HBSS bathing the cells to achieve the desired final concentrations used in the experiment. Indicator fluorescence was excited at 488 nm; fluorescence images were acquired with a cooled CCD camera. [J. Kao, Univ. of Maryland Medical School]

Figure 4 Methods →

REF52 fibroblasts were incubated at room temperature for 60 minutes with 2 µM ION Natrium Green-2 AM ester in bicarbonate-buffered DMEM, supplemented with 10% fetal bovine serum, under an atmosphere of 5% CO₂/95% O₂; the medium also contained 0.0075% (w/v) of the nonionic surfactant, Pluronic F-127. The cells were then transferred into 145 mM NMG gluconate (pH 7.4). Amphotericin B was prepared as a 50 mM solution in DMSO; 1000-fold dilution into aqueous medium gave the desired final concentration used in the experiment. Appropriate volumes of 4 M NaCl or KCl were added to achieved the desired concentrations in the aqueous medium. Indicator fluorescence was excited at 488 nm; fluorescence images were acquired with a cooled CCD camera. [J. Kao, Univ. of Maryland Medical School]

ING-2 Response to Na⁺ Influx Facilitated by SQI-Pr

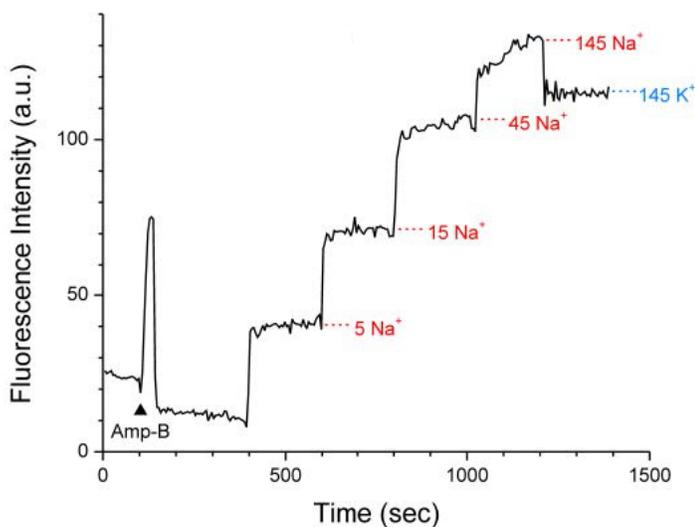
Plot quantifying the enhanced fluorescence of ING-2, resulting from increased cytosolic sodium ion concentration provoked by the application of the sodium ionophore SQI-Pr. REF52 fibroblasts loaded with ING-2 (AM) in HBSS were exposed to 40 µM SQI-Pr, a Na⁺ ionophore that promotes Na⁺/H⁺ exchange across the cell membrane. The fluorescence of intracellular ING-2 increased steadily, reflecting a rise in intracellular [Na⁺]. Further addition of 20 µM amphotericin B, a polyene natural product that increases membrane permeability to all the common monovalent ions, gave a small additional increment of indicator fluorescence, as expected. This experiment demonstrates the utility of ING-2 as a sodium indicator and the efficacy of SQI-Pr as a sodium ionophore. The response shown is an average from 30 cells. Experimental results courtesy of Dr. JPY Kao, University of Maryland



ING-2 Intracellular Titration with NaCl

Plot quantifying the enhanced fluorescence of ING-2, resulting from increased cytosolic sodium ion concentration. REF52 fibroblasts loaded with ING-2 (AM) were maintained in 145 mM N-methyl-D-glucamine (NMG) gluconate. To deplete cells of Na^+ and K^+ , the cells were treated with 50 μM amphotericin B, a polyene natural product that increases membrane permeability to all the common monovalent ions. Increments of NaCl were added to raise the sodium concentration in the extracellular medium to various levels (5, 15, 45, and 145 mM). After each increment, as the intracellular and extracellular $[\text{Na}^+]$ equilibrated, a corresponding increase in intracellular indicator fluorescence was observed. At the end of the experiment, an aliquot of KCl was added to raise $[\text{K}^+]$ to 145 mM. The added K^+ decreased the fluorescence of ING-2 modestly. The response shown is an average from 25 cells.

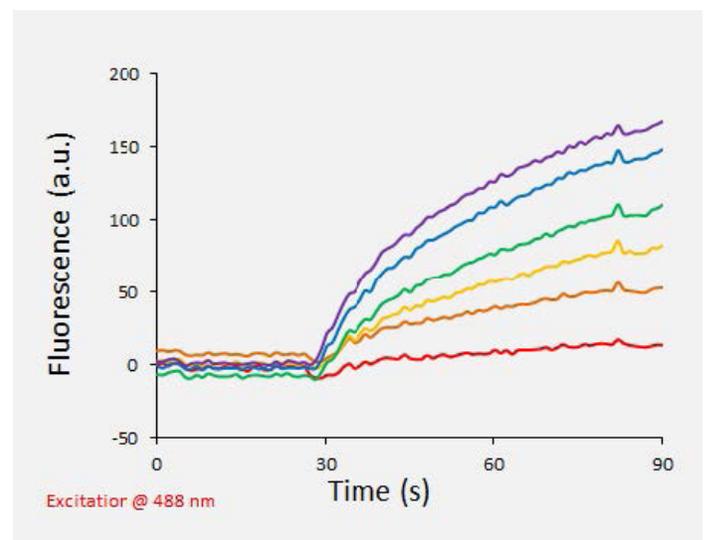
Experimental results courtesy of Dr. JPY Kao, University of Maryland



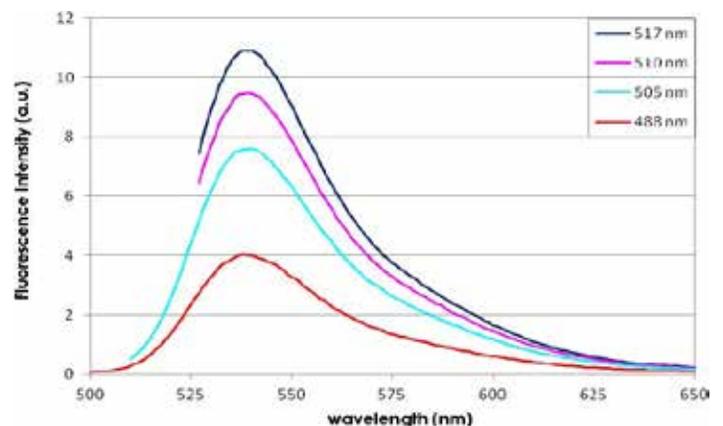
ING-2 Response to Voltage-Gated Sodium Channel Stimulation

Nav1.3-expressing HEK-293 cells loaded with ING-2 (AM) were exposed to a concentration series of lidocaine, a voltage-gated sodium channel blocker. The cells were stimulated with 25 mM veratridine in 20 mM HHBS.

Experimental results courtesy of Dr. Dave Weaver, Vanderbilt University



Effect of Excitation Wavelength on ING-2 Emission Intensity



Results

Figure 3

Response of intracellular ING-2 to Na⁺ influx facilitated by SQI-Pr

REF52 fibroblasts loaded with ING-2 (through incubation with AM ester) were maintained in Hanks' Balanced Salt Solution (HBSS). SQI-Pr is a Na⁺ ionophore that promotes Na⁺/H⁺ exchange across the cell membrane, and thus causes Na⁺ influx. Upon application of 40 µM SQI-Pr, the fluorescence of intracellularly-loaded ING-2 increased steadily, reflecting a rise in intracellular [Na⁺]. Further addition of 20 µM amphotericin B, a polyene natural product that increases membrane permeability to all the common monovalent ions (Na⁺, K⁺, H⁺ and Cl⁻), gave a small additional increment of indicator fluorescence, as expected. This experiment demonstrates the utility of ING-2 as a sodium indicator, and the efficacy of SQI-Pr as a sodium ionophore. The response shown is the average from 30 cells. [J. Kao, Univ. of Maryland Medical School]

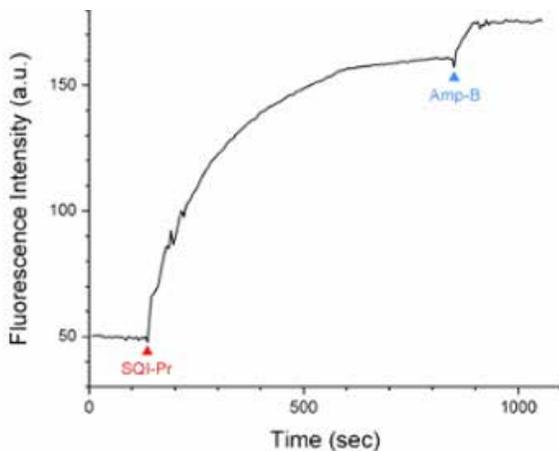
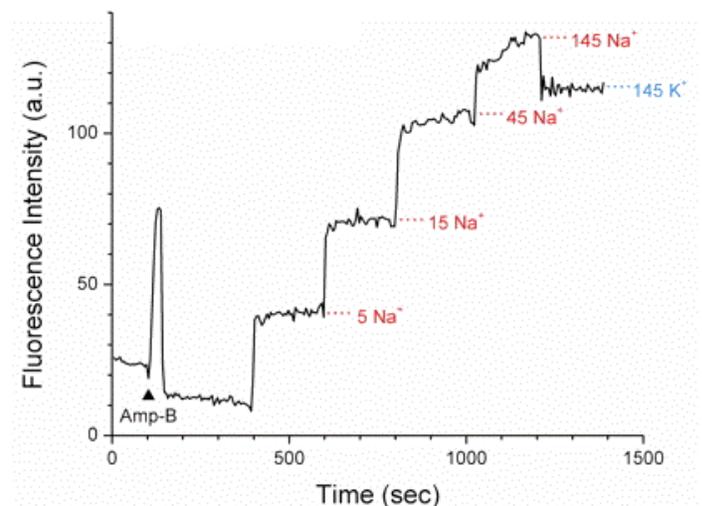


Figure 4

Response of intracellular ING-2 to Na⁺ influx

REF52 fibroblasts were loaded with ING-2 (through incubation with AM ester) and maintained in 145 mM N-methyl-D-glucamine (NMG) gluconate. To deplete cells of Na⁺ and K⁺, the cells were treated with 50 µM amphotericin-B (a polyene microbial metabolite that markedly increases membrane permeability to monovalent ions; e.g., Na⁺, K⁺, H⁺ and Cl⁻). Increments of NaCl were added to raise sodium concentration ([Na⁺]) in the extracellular medium to various levels (5, 15, 45, and 145 mM, as indicated in the figure). After each increment, as the intracellular and extracellular [Na⁺] equilibrated, a corresponding increase in intracellular indicator fluorescence was observed. At the end of the experiment, an aliquot of KCl was added to raise [K⁺] to 145 mM (typical cytosolic value). The added K⁺ actually decreased the fluorescence of ING-2 modestly. The response shown is the average from 25 cells. [J. Kao, Univ. of Maryland Medical School]



ING-2 (AM) cell loading procedure for the experiments of Figures 2.6 and 2.7: (This procedure should not be treated as a general loading protocol for ING-2)

- (i) 2 μM of ING-2 AM is an average concentration.
- (ii) The sample is prepared with ~ 75 ppm Pluronic for dispersing the dye into the incubator buffer.
- (iii) The buffer consisted of HCO_3^- -DMEM with 10% fetal bovine serum (FBS) and kept under 5% CO_2 atmosphere.
- (iv) Loading time varied from 45 minutes to 70 minutes.
- (v) The experiments were conducted in HBSS.

Figure 2.6

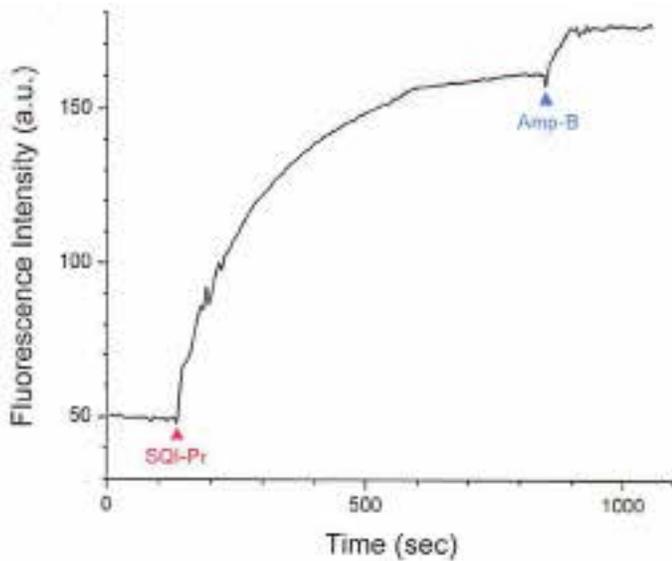
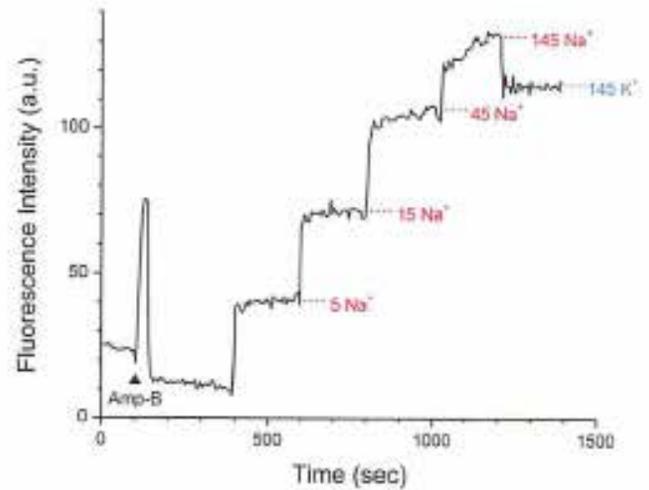


Figure 2.7



[J. Kao of Univ. of Maryland Medical School]

Loading Protocol

A sample loading procedure may be accessed in Merck Research Laboratories' SLAS 2011 poster by O'Donnell, et al., for ING-2 in a 1536-well voltage-gated sodium channel assay.

The following is supplied as a starting point for non-invasive loading of ING-2 via the acetoxymethyl (AM) ester version. Due to cell type and other experimental variations, loading conditions will require optimization.

1. Prepare a 1 mM stock solution of ING-2 AM in anhydrous dimethylsulfoxide (DMSO). (A more concentrated solution may be used to minimize final DMSO percentage.)
 - ◀ For a 500 µg vial, dissolve the contents of the vial in 460 µL DMSO.
 - ◀ For a 50 µg vial, use 46 µL DMSO.
2. For a 500µg vial, divide the stock solution into aliquots that will be consumed per experiment.
 - ◀ Store the aliquots at -20 °C, protected from moisture and light.
 - ◀ Repeated freezing and thawing of a stock solution typically leads to degradation of the product via hydrolysis of the AM esters
 - ◀ For your convenience, we supply ING-2 (AM) in 50 µg packages.
3. Dilute the stock solution to twice the original volume with a solution of 20% Pluronic F-127 in DMSO.
4. Disperse the ING-2/Pluronic F-127 solution into 100 times the volume of serumfree culture medium.
 - ◀ The final ING-2 (AM) concentration is 5 µM. (The concentration of ING-2 AM may require optimization.)
 - ◀ Pluronic F-127 is 0.1% of the final solution. (The final concentration of Pluronic F-127 may require optimization.)
5. Incubate the cells for one hour at room temperature. (Incubation time and temperature, 20°C vs. 37°C, may also require optimization.)
6. Remove the cell loading medium and wash the cells with serum-free and dye-free medium.
7. The cells are now loaded with ING-2 AM and ready for your sodium imaging experiments.

Safety Data Sheet

ION NaTRIUM Green-2 AM



SECTION 1: Identification of the Substances and the Company/Undertaking

Identification of the Substance or Mixture

Catlog Numbers: 2011C | 2011F

Product Name: ION NaTRIUM Green-2 AM

Company/Undertaking Identification

Ion Biosciences
3055 Hunter Road, Box 3
San Marcos, TX 78666
+1 512.957.9123

24 hour Emergency Response

866-536-0631

301-431-8585

+1-301-431-8585 (Outside of the U.S.)

For Research Use Only. Not for use in diagnostic procedures.

Section 2: Hazards Identification

GHS - Classification

Signal word: None

Health hazards: Not classified

Hazard statements: Not applicable

Precautionary Statements

Prevention: Not applicable

Response: Not applicable

Storage: Not applicable

Disposal: Not applicable

Principle Routes of Exposure

Potential Health Effects

Eyes: May cause eye irritation with susceptible persons.

Skin: May cause skin irritation in susceptible persons.

Inhalation: May be harmful by inhalation.

Ingestion: May be harmful if swallowed.

Specific Effects

Carcinogenic effects: No information available.

Mutagenic effects: No information available.

Reproductive toxicity: No information available.

Sensitization: No information available.

Target organ effects: No known effects under normal use conditions.

HMIS

Health	0
Flammability	0
Reactivity	0

Section 3: Composition/Information on Ingredients

The product contains no substances which at their given concentration, are considered to be hazardous to health.

Section 4: First Aid Measures

Skin contact: Rinse cautiously with water for several minutes.

Immediate medical attention is not required.

Eye contact: Rinse cautiously with water for several minutes.

Remove contact lenses, if present and easy to do.

Most important symptoms and effects, both acute and delayed:

Not applicable

Ingestion: Not expected to present a significant ingestion hazard

under anticipated conditions of normal use. If you feel unwell, seek medical advice. inhalation Not expected to be an inhalation hazard under anticipated conditions of normal use of this material. Consult a physician if necessary.

Notes to physician: Treat symptomatically.

Section 5: Firefighting Measures

Extinguishing Media

Suitable extinguishing media: Water spray. Carbon dioxide (CO₂). Foam. Dry chemical.

Unsuitable extinguishing media: Not Known

Specific hazards arising from the chemical: Not known

Advice for firefighters: Standard procedure for chemical fires.

Section 6: Accidental Release Measures

Personal precautions, protective equipment and emergency procedures:

Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. See Section 8 for more detail.

Environmental precautions: Avoid discharge into drains and

waterways whenever possible.

Methods and material for containment and cleaning up: Take up mechanically, placing in appropriate containers for disposal.

Reference to other sections: See section 8 and 12 for more information.

Section 7: Handling and Storage

Handling: Always wear recommended Personal Protective Equipment. No special handling advices are necessary.

Conditions for safe storage, including any incompatibilities: Store at -20°C. Protect from light and moisture.

Specific end use(s): For research use only.

Section 8: Exposure Controls/Personal Protection

Control Parameters

Exposure limits: We are not aware of any national exposure limit.

Engineering measures: Ensure adequate ventilation, especially in confined areas.

Exposure Controls

Personal Protective Equipment: Personal Protective Equipment requirements are dependent on the user institution's risk assessment and are specific to the risk assessment for each laboratory where this material may be used.

Respiratory protection: In case of insufficient ventilation, wear suitable respiratory equipment.

Hand protection: Impervious gloves.

Eye protection: Wear safety glasses with side shields (or goggles).

Skin and body protection: Lightweight protective clothing.

Hygiene measures: Handle in accordance with good industrial hygiene and safety practice.

Environmental exposure controls: Prevent product from entering drains or waterways whenever possible.

Section 9: Physical and Chemical Properties

Information on basic physical and chemical properties.

Form: Solid

Appearance: No data available

Odor: No data available

Odor threshold: No data available

Boiling point/boiling range: °C No data available; °F No data available

Melting point/melting range: °C No data available; °F No data available

Flash point: °C No data available; °F No data available

Autoignition temperature: °C No data available; °F No data available

Oxidizing properties: No data available

Water solubility: No data available

Section 10: Stability and Reactivity

Reactivity: None known.

Stability: Stable under normal conditions.

Materials to avoid: No dangerous reaction known under conditions of normal use.

Possibility of hazardous reactions: Hazardous reaction has not been reported

Hazardous decomposition products: None under normal use conditions.

Polymerization: Hazardous polymerization does not occur.

Conditions to avoid: None under normal processing.

Hazardous decomposition products: Carbon monoxide (CO). Carbon dioxide (CO₂). Nitrogen oxides (NO_x). Hydrogen chloride.

Section 11: Toxicological Information

Acute Toxicity: To the best of our knowledge, the chemical, physical, biological, and toxicological properties of this product have not been thoroughly investigated.

Principle Routes of Exposure

Potential Health Effects

Eyes: May cause eye irritation with susceptible persons.

Skin: May cause skin irritation in susceptible persons.

Inhalation: May be harmful by inhalation.

Ingestion: May be harmful if swallowed.

Carcinogenic effects: No information available.

Mutagenic effects: No information available.

Reproductive toxicity: No information available.

Sensitization: No information available.

Section 12: Ecological Information

Ecotoxicity: The environmental impact of this product has not been fully investigated.

Mobility: No information available.

Biodegradation: No information available.

Bioaccumulation: No information available.

Section 13: Disposal Considerations

Waste treatment methods: The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique.

Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations

Section 14: Transport information

IATA/ADR/DOT-US/IMDG: Not Classified as dangerous in the meaning of transport regulations.

Proper shipping name: No dangerous good in sense of these transport regulations

Hazard class: None

Subsidiary class: None

Packing group: None

UN-No: None

Environmental hazards: None

Section 15: Regulatory Information

US Federal Regulations

SARA 313: This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61): This product does not contains HAPs.

US State Regulations

California Proposition 65: This product does not contain any Proposition 65 chemicals.

WHMIS Hazard Class: Non-controlled

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

Section 16: Other Information

For Research Use Only. Not for use in diagnostic procedures.

"The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein.

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