

Store at
-20°C

#42239

CellSimple™ Mitochondrial Membrane Potential Assay Kit (I)

1 Kit (100 assays)



Cell Signaling
TECHNOLOGY®

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orders@cellsignal.com

Rev. 12/07/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Storage Temp.
JC-1	14121	3 x 15 µg	-20°C
CCCP	13550	1 x 100 µl	-20°C
Phosphate Buffered Saline (PBS-20X)	9808	1 x 25 ml	RT

Description: CellSimple™ Mitochondrial Membrane Potential Assay Kit (I) is a fluorescent assay designed for use with the CellSimple™ Cell Analyzer. It detects the mitochondrial membrane potential in living cells. The kit includes the cationic dye JC-1 and a mitochondrial membrane potential disruptor CCCP (carbonyl cyanide 3-chlorophenylhydrazone). JC-1 is a cell membrane permeable, fluorescent dye with green emission (~520 nm). When JC-1 accumulates in intact mitochondria, the dye forms aggregates that lead to orange-red fluorescence (~590 nm). The mean fluorescence intensity (MFI) of the orange-red emission can be used as an indicator for mitochondrial membrane potential.

Background: Mitochondria function as the main cellular powerhouse and play important roles in other processes, such as steroid metabolism, calcium homeostasis, apoptosis, and cellular proliferation. Mitochondrial membrane potential is a key indicator of mitochondrial function and cell health (1,2). The dissipation of mitochondrial membrane potential is considered an early indicator of apoptosis (3).

JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylimidocarbocyanine iodide) is a cell membrane permeable, cationic dye. In normal cells, JC-1 concentrates in mitochondria to form aggregates in response to high membrane potential. Decreased mitochondrial membrane potential results in dispersal of mostly monomeric JC-1 throughout the cell. When excited at 490 nm, JC-1 monomers emit a green fluorescence with a maximum at ~520 nm. Aggregates of JC-1 emit an orange-red fluorescence with a maximum at ~590 nm. Therefore, the fluorescence intensity of the orange-red emission and the ratio of orange-red fluorescence to green fluorescence can be used to measure mitochondrial membrane potential and serve as an indicator of overall cell health (4).

CellSimple™ Cell Analysis System: The CellSimple™ Cell Analyzer is a benchtop instrument that utilizes a disposable thin-film cassette and a combination of a 488 nm laser, two photomultiplier tubes (525/45 nm and 561 nm LP filters), Coulter Principle-based cell measurements, and on-board software to provide easy-to-run applications and data analysis. Data acquisition occurs within approximately 10 seconds per test. The instrument relies on disposable cassettes for sample handling, which alleviates the need for flow cell cleaning and fluidics maintenance and the instrument is small enough to be portable between the lab bench and the hood. Applications include quantitative assessments of cell viability, apoptosis, other labeled antibody markers and single and multiplexed bead-based assays for protein and cellular analysis.

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature and left unused. Upon receipt, #9808 should be removed from kit box and stored at room temperature. *Remaining components should be stored at -20°C.*

Background References:

- (1) Perry, S.W. et al. (2011) *Biotechniques* 50, 98-115.
- (2) Nesti, C. et al. (2007) *Biosci Rep* 27, 165-71.
- (3) Petit, P.X. et al. (1995) *J Cell Biol* 130, 157-67.
- (4) Perelman, A. et al. (2012) *Cell Death Dis* 3, e430.

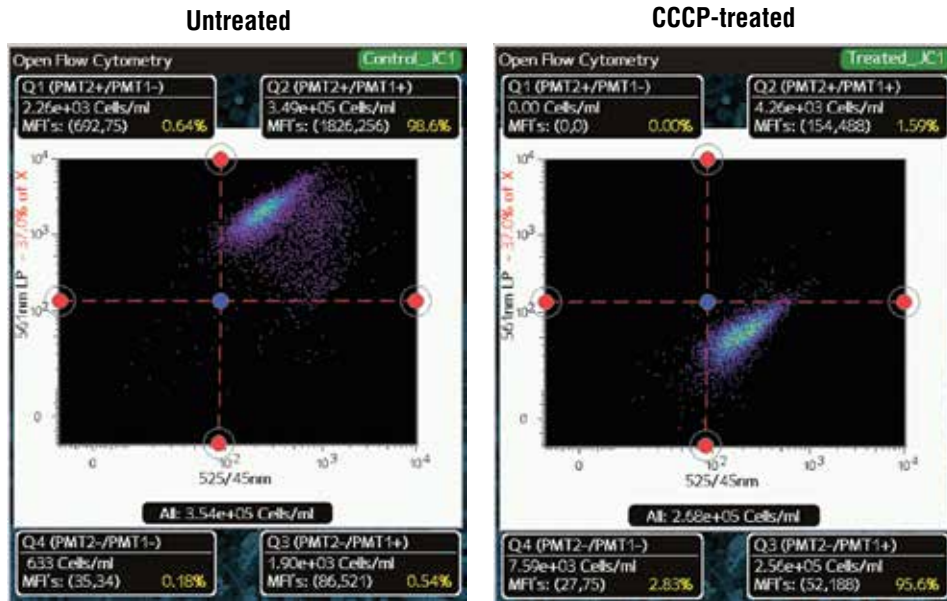
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



CellSimple™ cell-based analysis of live Jurkat cells untreated (left panel) or CCCP-treated (50 μM, 37°C, 15 min; right panel) and labeled with JC-1 (2 μM, 30 min) using the CellSimple™ Mitochondrial Membrane Potential Assay Kit (1). Data was collected in both red (561 nm LP) and green (525/45 nm) channels and analyzed on the Open Flow Cytometry application. Note the marked decrease in mean fluorescence intensity in the red channel upon CCCP treatment. Instrument screen shots are shown.

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Please note your screen may look slightly different from the screen shots on the data sheet due to variations between software versions.

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CellSimple™ Mitochondrial Membrane Potential Assay Kit (I) Protocol

- A. Instrumentation:** The CellSimple™ Mitochondrial Membrane Potential (I) Assay was specially designed for use with the CellSimple™ Cell Analyzer. However, either kit may be used with a flow cytometer or plate reader capable of providing excitation at approximately 480 nm and detecting fluorescent emission at approximately 520 nm and 590 nm.
- B. Kit components:**
- JC-1
 - CCCP
 - Phosphate Buffered Saline (PBS-20X)
- C. Additional reagents needed, but not supplied.**
- DMSO
 - Reverse osmosis/deionized (RO/DI) water or equivalent
- D. Reagent preparation**
- 1. 1X PBS:** To prepare 1 L 1X PBS add 25 ml PBS-20X to 475 ml RO/DI water, mix.
Note: For flow cytometry application, adding 0.5% BSA to 1X PBS buffer may help to prevent cell loss.
 - 2. JC-1 Stock Solution:** Add 110 µl DMSO to each vial of JC-1 to make a 200 µM stock solution.
 - 3. CCCP:** Allow the 50 mM CCCP solution to equilibrate to room temperature before use.
- E. Protocol for suspension cells**
1. Suspend cells in warm media or PBS at 1×10^6 cell/ml. Prepare 1 ml aliquots; each 1 ml cell aliquot is one assay point. Make sure there are enough cells for your experiment. For example, if one compound is going to be assayed at three different concentrations, a total of 4×1 ml samples will be needed (this includes a positive control).
 2. Add test compound(s) to sample tubes at desired concentration and incubate cells for desired time. For best results, a compound titration and incubation time course can help to determine the best assay conditions. To prepare the positive control (mitochondrial membrane potential loss), add 1 µl of 50 mM CCCP to the control tube for a 50 µM final concentration; incubate cells at 37°C for 15 min.
 3. Add 10 µl of 200 µM JC-1 stock solution to each sample (2.0 µM final concentration). Incubate cells in an incubator (37°C and 5% CO₂) for 15 to 30 min.
 4. Centrifuge sample at 300 x g for 5 min then remove the supernatant.
 5. Wash cells once with 1.0 ml warm 1X PBS wash buffer. Repeat step 7.
 6. Re-suspend cells into 1.0 ml warm 1X PBS.
 7. Analyze sample using an appropriate instrument.
 - i. For analysis using the CellSimple™ Cell Analyzer use the Open Flow Cytometry Application selecting only the 561 nm LP detection channel. Results may be obtained by choosing the histogram feature (X/Y function on the display screen) or by selecting the control and treated samples in the save data files and using the overlay file feature. Please see the CellSimple user guide for more details about using the Open Flow Application.
 - ii. If samples are to be analyzed on a plate reader, transfer 100 µl/cell suspension/well to a black 96 well plate with a clear bottom and read using the following settings: excitation at approximately 550 nm and emission at approximately 580 nm.
 - iii. Analyze sample on flow cytometer with excitation of ex550/em580
 8. To calculate the red to green ratios using the controls:
(Red (MFI) control÷Green (MFI) control):(Red (MFI) sample÷Green (MFI) sample)
- F. Protocol for adherent cells**
1. Plate cells in a 96 well plate in warm culture medium and place in incubator overnight to allow cells to attach to the plate. A typical cell number is between 1×10^4 and 5×10^4 cells/well. A cell number titration may be necessary for optimal results.
 2. Aspirate media from the plate and add test compounds in growth medium or 1X PBS to plate at 100 µl/well and incubate cells for desired time. Compound titration and incubation time course can help determine the best assay conditions. For a positive control (mitochondrial membrane potential loss), add CCCP to the control wells at 50 µM final concentration and incubate cells at 37°C for 15 min. For example, add 1 µl of 50 mM stock CCCP to 100 µl medium to make 500 µM CCCP; then add 10 µl of this 500 µM CCCP to each well containing 100 µl medium to get final concentration of 50 µM.
 3. Add 1 µl of JC-1 stock (200 µM) to each well to get a final concentration of 2 µM and place the plate in an incubator (37°C and 5% CO₂) for 15 to 30 min.
Note: JC-1 can be diluted 1:10 in media to make a 20 µM solution, add 10 µl of 20 µM JC-1 to each well containing 100 µl media for a final concentration of 2 µM.
 4. Aspirate the solution from the plate.
 5. Wash plate 3 times with warm 1X PBS and then add 1X PBS at 100 µl/well to the plate.
 6. Analyze sample using an appropriate instrument.
 - i. For analysis using the CellSimple™ Cell Analyzer use the Open Flow Cytometry Application selecting only the 561 nm LP detection channel. Results may be obtained by choosing the histogram feature (X/Y function on the display screen) or by selecting the control and treated samples in the save data files and using the overlay file feature. Please see the CellSimple user guide for more details about using the Open Flow Application.
 - ii. If samples are to be analyzed on a plate reader, transfer 100 µl/cell suspension/well to a black 96-well plate with a clear bottom and read using the following settings: excitation at approximately 550 nm and emission at approximately 580 nm.
 - iii. Analyze sample on flow cytometer with excitation of ex550/em580
 7. To calculate the red to green ratios using the controls:
(Red (MFI) control÷Green (MFI) control):(Red (MFI) sample÷Green (MFI) sample)



Safety Data Sheet (SDS) According to the OSHA Hazard Communication Standard 29 CFR 1910.1200
 Issuing Date: 2014-02-24 Revision Date: 2014-02-24 Version: 1

SECTION 1. Identification

Product identifier

Product number 9808
 Product name Phosphate Buffered Saline (PBS-20X)
 Other means of identification 9808BC, 9808P, 9808P2, 9808S

Recommended use of the chemical and restrictions on use

Identified uses This product is intended for research purposes only.
 Uses advised against This product is not intended for use in diagnostic procedures or therapeutics.
 This product is not intended for use in humans or animals.

Manufacturer, importer, supplier

Manufacturer address Cell Signaling Technology, Inc.
 3 Trask Lane
 Danvers, MA 01923
 United States
 TEL: +1 978 867 2300
 FAX: +1 978 867 2400
 www.cellsignal.com
 Website
 Email address support@cellsignal.com
 Emergency telephone number In case of emergency call CHEMTREC 1-800-424-9300

SECTION 2. Hazard(s) identification

Classification

This substance/mixture is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Serious eye damage/eye irritation Category 2B

GHS Label elements, including precautionary statements

Signal Word
 Warning

Hazard statement(s)
 Causes eye irritation.

Precautionary Statement(s)
 Wash face, hands and any exposed skin thoroughly after handling.
 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.

Supplementary Hazard Information
 No information available.

SECTION 3. Composition/information on ingredients

Chemical nature Aqueous buffer solution

Chemical Name	CAS No	Weight %
sodium chloride	7647-14-5	10-30

SECTION 4. First-aid measures

Eye contact Rinse thoroughly with plenty of water for at least 15 minutes, lifting lower and upper eyelids. Consult a physician.
 Skin contact Wash skin with soap and water.
 Inhalation Move to fresh air.
 Ingestion If swallowed, do not induce vomiting - seek medical advice.

Most important symptoms and effects, both acute and delayed

No information available.

Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

Advice for emergency responders

General advice For further assistance, contact your local Poison Control Center.
 Protection of first-aiders Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves.

SECTION 5. Fire-fighting measures

Extinguishing media

Suitable Extinguishing Media Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.
 Unsuitable Extinguishing Media CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical

No information available.

Explosion Data

Sensitivity to Mechanical Impact None.
 Sensitivity to Static Discharge None.

Protective Equipment and Precautions for Firefighters

As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

SECTION 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel Ensure adequate ventilation.
 Other information No information available.

Environmental precautions

See Section 12 for additional information.

Methods and material for containment and cleaning up

Methods for containment Prevent further leakage or spillage if safe to do so.
 Methods for cleaning up Pick up and transfer to properly labeled containers.

SECTION 7. Handling and storage

Precautions for safe handling

Handle in accordance with good industrial hygiene and safety practice.

Conditions for safe storage, including any incompatibilities

Technical measures/Storage conditions Keep containers tightly closed in a dry, cool and well-ventilated place.
 Packaging material No information available.
 Incompatible products None known based on information supplied.

SECTION 8. Exposure controls/personal protection

Control parameters

This product, as supplied, does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Appropriate engineering controls

Showers, eyewash stations, and ventilation systems.

Individual protection measures, such as personal protective equipment

Personal protective equipment (PPE) needs to be selected depending on the implemented engineering controls, frequency/duration of work activities and the concentrations of the hazardous substance.

Eye/face protection Safety glasses with side-shields.
 Skin and body protection Wear protective gloves/clothing.
 Respiratory protection If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.
 Hygiene measures Handle in accordance with good industrial hygiene and safety practice.

SECTION 9. Physical and chemical properties

Information on basic physical and chemical properties

Physical state Liquid
 Appearance No information available
 Color Colorless
 Odor No information available
 Odor Threshold No information available
 pH 7.4
 Melting point/freezing point No information available
 Initial boiling point and boiling range No information available
 Flash point No information available
 Evaporation rate No information available
 Flammability (solid, gas) No information available
 Upper flammability limit No information available.

Lower flammability limit No information available.
 Vapor pressure No information available.
 Vapor density No information available.
 Relative density No information available.
 Solubility No information available.
 Solubility in other solvents No information available.
 Partition coefficient: n-octanol/water No information available.
 Autoignition temperature No information available.
 Decomposition temperature No information available.
 Explosive properties No information available.
 Oxidizing properties No information available.
 VOC content No information available.
 Viscosity No information available.
 Density No information available.
 Solubility in other solvents No information available.

SECTION 10. Stability and reactivity

Reactivity

No information available.

Chemical stability

Stable under recommended storage conditions.

Possibility of hazardous reactions

Hazardous reactions None under normal processing.
 Hazardous polymerization None under normal processing.

Conditions to Avoid

No information available.

Incompatible Materials

None known based on information supplied.

Hazardous Decomposition Products

None known based on information supplied.

SECTION 11. Toxicological information

Information on likely routes of exposure

Inhalation There is no data available for this product.
 Eye contact May cause temporary eye irritation.
 Skin contact There is no data available for this product.
 Ingestion There is no data available for this product.

Information on toxicological effects

This material should only be handled by, or under the close supervision of, those properly qualified in the handling and use of potentially hazardous chemicals. It should be borne in mind that the toxicological and physiological properties of this compound is not well defined.

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
sodium chloride	3000 mg/kg (Rat)	10 g/kg (Rabbit)	42 g/m ³ (Rat) 1 h

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Symptoms	No information available.
Sensitization	No information available.
Mutagenic effects	No information available.
Carcinogenicity	No component of this product present at levels greater than or equal to 0.1% is identifiable as probable, possible or confirmed carcinogen by IARC, ACGIH, NTP, or OSHA.
Reproductive toxicity	No information available.
STOT - single exposure	No information available.
STOT - repeated exposure	No information available.
Neurological effects	No information available.
Aspiration Hazard	No information available.

SECTION 12. Ecological information**Ecotoxicity**

Chemical Name	Toxicity to algae	Toxicity to fish	Toxicity to daphnia and other aquatic invertebrates
sodium chloride	-	LC50 5560 - 6080 mg/L (Lepomis macrochirus) 96 h LC50 12946 mg/L (Lepomis macrochirus) 96 h LC50 4747 - 7624 mg/L (Oncorhynchus mykiss) 96 h LC50 7050 mg/L (Pimephales promelas) 96 h LC50 6420 - 6700 mg/L (Pimephales promelas) 96 h LC50 6020 - 7070 mg/L (Pimephales promelas) 96 h	EC50 340.7 - 469.2 mg/L (Daphnia magna) 48 h EC50 1000 mg/L (Daphnia magna) 48 h

Persistence and degradability	No information available.
Bioaccumulation	No information available.
Mobility	No information available.

Other adverse effects

No information available.

SECTION 13. Disposal considerations**Waste Disposal Methods**

Dispose of in accordance with all applicable national environmental laws and regulations.

Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

SECTION 14. Transport information

This material is not subject to regulation as a hazardous material for shipping.

SECTION 15. Regulatory information**North American Inventory Listing**

Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL

SECTION 16. Other information

Issuing Date: 2014-02-24
 Revision Date: 2014-02-24
 Disclaimer:

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet

sodium chloride	Listed	Not Listed	Listed	Not Listed
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Canadian Workplace Hazardous Materials Information System (WHMIS) Classification

	Class D2B - Toxic Material at >= 1%
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SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazard Categories

Acute Health Hazard	Yes
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act

This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

CERCLA

This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

California Proposition 65

This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

This product contains the following U.S. State Right to Know chemicals:

Chemical Name	New Jersey	Massachusetts	Pennsylvania
disodium hydrogenorthophosphate	Listed	Listed	Listed

U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

US Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.



Safety Data Sheet (SDS) According to the OSHA Hazard Communication Standard 29 CFR 1910.1200
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SECTION 1. Identification**Product identifier**

Product number	13550
Product name	CCCP
Other means of identification	13550M, 13550S

Recommended use of the chemical and restrictions on use

Identified uses	This product is intended for research purposes only.
Uses advised against	This product is not intended for use in diagnostic procedures or therapeutics. This product is not intended for use in humans or animals.

Manufacturer, importer, supplier

Manufacturer address	Cell Signaling Technology, Inc. 3 Trask Lane Danvers, MA 01923 United States TEL: +1 978 867 2300 FAX: +1 978 867 2400 www.cellsignal.com
Website	www.cellsignal.com
Email address	support@cellsignal.com
Emergency telephone number	In case of emergency call CHEMTREC 1-800-424-9300

SECTION 2. Hazard(s) identification**Classification**

This substance/mixture is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Skin corrosion/irritation	Category 2
Serious eye damage/eye irritation	Category 2
Flammable liquids	

GHS Label elements, including precautionary statements

Signal Word
Warning

Hazard statement(s)
Combustible liquid.
Causes skin irritation. Causes serious eye irritation.

Precautionary Statement(s)

Wash face, hands and any exposed skin thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
 IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash before reuse.
 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Supplementary Hazard Information

No information available.

SECTION 3. Composition/information on ingredients

Chemical nature Liquid solution containing an inorganic compound

Chemical Name	CAS No	Weight %
[(3-chlorophenyl)hydrazono]malononitrile	555-60-2	0.5-1.5
dimethyl sulfoxide	67-68-5	60-100

SECTION 4. First-aid measures

Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. Keep eye wide open while rinsing.
Skin contact	Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes. Immediate medical attention is not required. If symptoms persist, call a physician.
Inhalation	Move to fresh air. Consult a physician. If not breathing, give artificial respiration. Move to fresh air in case of accidental inhalation of vapors. Immediate medical attention is not required. If symptoms persist, call a physician. IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing.
Ingestion	Rinse mouth. Drink plenty of water. If symptoms persist, call a physician. Do NOT induce vomiting. Clean mouth with water. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person. Consult a physician. If swallowed, do not induce vomiting - seek medical advice.

Most important symptoms and effects, both acute and delayed

No information available.

Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

Advice for emergency responders

General advice	If symptoms persist, call a physician. Show this safety data sheet to the doctor in attendance. Do not breathe dust/fume/gas/mist/vapors/spray.
Protection of first-aiders	Use personal protective equipment.

SECTION 5. Fire-fighting measures**Extinguishing media**

Suitable Extinguishing Media Cool containers / tanks with water spray. Use: Dry chemical. Carbon dioxide (CO₂). Water spray. Alcohol-resistant foam.

Unsuitable Extinguishing Media CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical

Keep product and empty container away from heat and sources of ignition. Risk of ignition.

Explosion Data

(vacated) = Vacated limits revoked by the Court of Appeals decision in AFL-CIO v. OSHA, 965 F.2d 962 (11th Cir., 1992).

Appropriate engineering controls

Showers, eyewash stations, and ventilation systems.

Individual protection measures, such as personal protective equipment

Personal protective equipment (PPE) needs to be selected depending on the implemented engineering controls, frequency/duration of work activities and the concentrations of the hazardous substance.

Eye/face protection	Tightly fitting safety goggles.
Skin and body protection	Wear protective gloves/clothing.
Respiratory protection	If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.
Hygiene measures	When using, do not eat, drink or smoke. Provide regular cleaning of equipment, work area and clothing.

SECTION 9. Physical and chemical properties**Information on basic physical and chemical properties**

Physical state	Liquid
Appearance	No information available
Color	Yellow
Odor	Sulphurous
Odor Threshold	No information available
pH	No information available
Melting point/freezing point	16 - 19 °C / 60.8 - 66.2 °F
Initial boiling point and boiling range	189 °C / 372.2 °F
Flash point	87 °C / 188.6 °F Closed cup
Evaporation rate	No information available
Flammability (solid, gas)	No information available
Upper flammability limit	42%
Lower flammability limit	3.5%
Vapor pressure	0.55 hPa @ 20 °C
Vapor density	2.7
Relative density	1.1 g/ml
Solubility	Completely soluble
Solubility in other solvents	No information available
Partition coefficient: n-octanol/waterlog	Pow: -2.03
Autoignition temperature	No information available
Decomposition temperature	No information available.
Explosive properties	No information available
Oxidizing properties	No information available
VOC content	No information available
Viscosity	No information available.
Density	No information available.
Solubility in other solvents	No information available

SECTION 10. Stability and reactivity**Reactivity**

No information available.

Chemical stability

Stable under recommended storage conditions.

Sensitivity to Mechanical Impact None.
Sensitivity to Static Discharge None.

Protective Equipment and Precautions for Firefighters

As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

SECTION 6. Accidental release measures**Personal precautions, protective equipment and emergency procedures**

For non-emergency personnel	Remove all sources of ignition. Use personal protective equipment. Take precautionary measures against static discharges. Heat, flames and sparks. Ensure adequate ventilation.
Other information	No information available.

Environmental precautions

Do not flush into surface water or sanitary sewer system. Prevention of fire and explosion. A vapor suppressing foam may be used to reduce vapors. Try to prevent the material from entering drains or water courses. Do not allow material to contaminate ground water system. Most vapors are heavier than air. They will spread along ground and collect in low or confined areas (sewers, basements, tanks). Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas.

Methods and material for containment and cleaning up

Methods for containment	Prevent further leakage or spillage if safe to do so.
Methods for cleaning up	Cover liquid spill with sand, earth or other noncombustible absorbent material. Cover powder spill with plastic sheet or tarp to minimize spreading. Pick up and transfer to properly labeled containers. Soak up with inert absorbent material. Dam up. Take precautionary measures against static discharges.

SECTION 7. Handling and storage**Precautions for safe handling**

Avoid contact with skin, eyes and clothing. Wear personal protective equipment. Prevent the formation of vapors, mists and aerosols. Do not eat, drink or smoke when using this product. There is a hazard associated with rags, paper or any other material used to remove spills which become soaked with product. Avoid accumulation of these: they are to be disposed off safely after use. Avoid static electricity build up with connection to earth. Use only in area provided with appropriate exhaust ventilation. To avoid ignition of vapors by static electricity discharge, all metal parts of the equipment must be grounded. Keep away from open flames, hot surfaces and sources of ignition.

Conditions for safe storage, including any incompatibilities

Technical measures/Storage conditions	Keep away from direct sunlight.
Packaging material	No information available.
Incompatible products	Strong oxidizing agents. Acyl, aryl, and nonmetal halides. Boron compounds. Metal salts of oxoacids.

SECTION 8. Exposure controls/personal protection**Control parameters**

Chemical Name	Occupational exposure limit values		
	ACGIH TLV	OSHA PEL	NIOSH REL
[(3-chlorophenyl)hydrazono]malononitrile	-	TWA : 5 mg/m ³ S*	IDLH : 25 mg/m ³

Possibility of hazardous reactions

Hazardous reactions	None under normal processing. Vapors may form explosive mixtures with air. 10.5.
Hazardous polymerization	Incompatible materials. None under normal processing.

Conditions to Avoid

Heating in air.

Incompatible Materials

Strong oxidizing agents. Acyl, aryl, and nonmetal halides. Boron compounds. Metal salts of oxoacids.

Hazardous Decomposition Products

Thermal decomposition can lead to release of irritating gases and vapors: Sulfur oxides.

SECTION 11. Toxicological information**Information on likely routes of exposure**

Inhalation	There is no data available for this product.
Eye contact	Irritating to eyes.
Skin contact	Irritating to skin. Components of this product may be absorbed into the body through the skin.
Ingestion	There is no data available for this product.

Information on toxicological effects

This material should only be handled by, or under the close supervision of, those properly qualified in the handling and use of potentially hazardous chemicals. It should be borne in mind that the toxicological and physiological properties of this compound is not well defined.

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
[(3-chlorophenyl)hydrazono]malononitrile	= 100 mg/kg (Rat)	= 300 mg/kg (Rat)	= 0.5 mg/l (Rat) Dust/mist
dimethyl sulfoxide	14500 mg/kg (Rat)	40000 mg/kg (Rat)	-

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Symptoms	No information available.
Sensitization	No information available.
Mutagenic effects	No information available.
Carcinogenicity	No component of this product present at levels greater than or equal to 0.1% is identifiable as probable, possible or confirmed carcinogen by IARC, ACGIH, NTP, or OSHA.
Reproductive toxicity	No information available.
STOT - single exposure	No information available.
STOT - repeated exposure	No information available.
Neurological effects	No information available.
Aspiration Hazard	No information available.

SECTION 12. Ecological information**Ecotoxicity**

1% of the mixture consists of components(s) of unknown hazards to the aquatic environment.

Chemical Name	Toxicity to algae	Toxicity to fish	Toxicity to daphnia and other aquatic invertebrates

dimethyl sulfoxide	EC50 12350 - 25500 mg/L (Skeletonema costatum) 96 h	LC50 40 g/L (Lepomis macrochirus) 96 h LC50 33 - 37 g/L (Oncorhynchus mykiss) 96 h LC50 34000 mg/L (Pimephales promelas) 96 h LC50 41.7 g/L (Cyprinus carpio) 96 h	EC50 7000 mg/L (Daphnia species) 24 h
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Persistence and degradability
No information available.
Bioaccumulation
No information available.
Mobility
No information available.

Chemical Name	Octanol-Water Partition Coefficient
dimethyl sulfoxide	-2.03

Other adverse effects**SECTION 13. Disposal considerations****Waste Disposal Methods**

Dispose of in accordance with all applicable national environmental laws and regulations.

Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

SECTION 14. Transport information

This material is not subject to regulation as a hazardous material for shipping.

SECTION 15. Regulatory information**North American Inventory Listing**

Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL
[(3-chlorophenyl)hydrazono]malononitrile	Listed	Not Listed	Not Listed	Listed
dimethyl sulfoxide	Listed	Not Listed	Listed	Not Listed

Canadian Workplace Hazardous Materials Information System (WHMIS) Classification

This product does not meet the criteria for classification under the Hazardous Products Act.

SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product contains a chemical or chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372:

Chemical Name	CAS No	SARA 313 - Threshold Values %
[(3-chlorophenyl)hydrazono]malononitrile	555-60-2	1.0

SARA 311/312 Hazard Categories

Acute Health Hazard Yes
Chronic Health Hazard No
Fire Hazard Yes
Sudden Release of Pressure Hazard No

Reactive Hazard No

Clean Water Act

This product contains the following substances which are regulated pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42):

Chemical Name	CWA - Reportable Quantities	CWA - Toxic Pollutants	CWA - Priority Pollutants	CWA - Hazardous Substances	CWA - Bioaccumulative Chemicals of Concern (BCCs)
[(3-chlorophenyl)hydrazono]malononitrile	Not Listed	Listed	Listed	Not Listed	Not Listed

CERCLA

This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

California Proposition 65

This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

This product does not contain any substances regulated under applicable state right-to-know regulations

Chemical Name	New Jersey	Massachusetts	Pennsylvania
[(3-chlorophenyl)hydrazono]malononitrile	Listed	Not Listed	Listed
dimethyl sulfoxide	Listed	Not Listed	Not Listed

U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

US Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.

SECTION 16. Other information

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Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet