

Store at
-20°C
#13296

Mitochondrial Membrane Potential Assay Kit (II)

www.cellsignal.com

Support: 877-678-TECH (8324)
info@cellsignal.com

Orders: 877-616-CELL (2355)
orders@cellsignal.com

rev. 11/13/17

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

Description: The Mitochondrial Membrane Potential Assay Kit (II) is a fluorescent assay that detects the mitochondrial membrane potential in living cells. The kit includes the cationic dye TMRE (tetramethylrhodamine ethyl ester perchlorate) and a mitochondrial membrane potential disruptor CCCP (carbonyl cyanide 3-chlorophenylhydrazone). TMRE is a cell membrane permeable, fluorescent dye that accumulates in intact mitochondria. Depolarized or inactive mitochondria exhibit decreased membrane potential, resulting in reduced TMRE accumulation.

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

TMRE (tetramethylrhodamine, ethyl ester) is a cell membrane permeable cationic dye. In normal cells, TMRE accumulates in the mitochondria in response to their high membrane potential and negative charge. When excited at 550 nm, TMRE emits an orange-red fluorescence with a maximum at 575 nm (orange-red). Cells that have lost membrane potential or mitochondria activity cannot accumulate TMRE. Therefore, the fluorescence intensity of the orange-red emission can be used to measure mitochondria membrane potential and is an indicator for cell health (4).

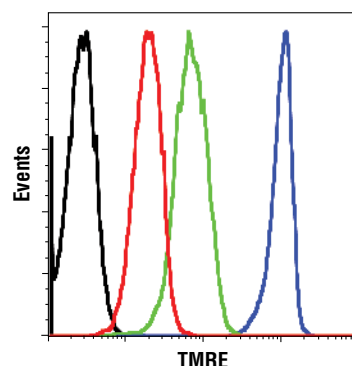
Specificity/Sensitivity: The Mitochondrial Membrane Potential Assay Kit (II) is expected to detect the mitochondrial membrane potential in living cells cross all species. For the best result, a cell number titration is recommended when using a plate-reader and a 96-well plate.

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) O'Reilly, C.M. et al. (2003) *Biophys J* 85, 3350-7.

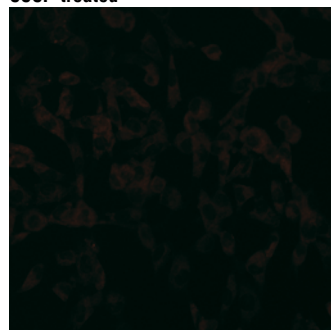
Products Included	Item	Quantity	Storage Temp
TMRE	13472	29 µg	-20°C
CCCP	13550	100 µl	-20°C
Phosphate Buffered Saline (PBS-20X)	9808	25 ml	Room Temp

Note: 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.

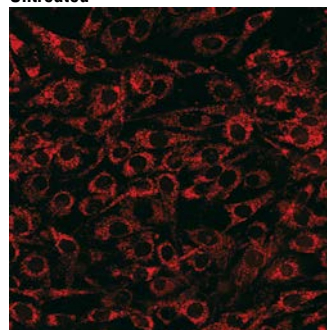


◀ Figure 1. Flow cytometric analysis of Jurkat cells, unlabeled (black) or labeled with 200 nM TMRE and treated with 0 µM CCCP (blue), 3.2 µM CCCP (green), or 80 µM CCCP (red).

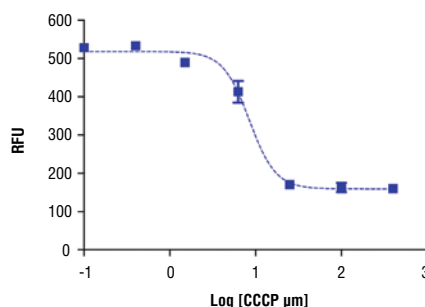
CCCP-treated



Untreated



◀ Figure 2. Confocal immunofluorescent analysis of NIH/3T3 cells (2x10⁶ cell/ml) seeded in a 96-well black plate with a clear bottom and incubated overnight. Cells were untreated, or treated with CCCP (400 µM, 20 min) followed by labeling with TMRE (200 nM, 30 min).



◀ Figure 3. HeLa cells (3x10⁶ cell/ml) were treated with various concentrations of CCCP for 15 minutes prior to labeling with 200 nM TMRE.

Thank you for your recent purchase. If you would like to provide a review visit www.cellsignal.com/comments.

© 2014 Cell Signaling Technology, Inc. Cell Signaling Technology® is a trademark of Cell Signaling Technology, Inc.
Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

 **Cell Signaling**
TECHNOLOGY®

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.

Mitochondrial Membrane Potential Assay Kit (II) Protocol

Instrumentation Required

1. Flow cytometer with excitation approximately 550 nm and emission 580 nm. Very good results can also be achieved with a common argon blue line laser (488 nm).
2. Plate reader that can read 96-well plates with excitation approximately 550 nm and emission approximately 580 nm
3. 96-well plate (black with clear bottom)

Reagent Preparation

1. Prepare 1X PBS by diluting 20X PBS (included in each kit) in reverse osmosis deionized (RODI) or equivalently purified water.
Note: For flow cytometry, adding 0.5% BSA to wash buffer may help to prevent cell loss during the process.
2. Add 55 μ l DMSO to each vial of TMRE to make a 1000 μ M stock solution. Each vial includes enough TMRE for five 96-well plates (0.1 μ l/well) or 50 flow cytometry assays (10 μ l/assay). Aliquot if desired and store at -20° C.
3. Dilute TMRE to 1:500 with full cell culture medium to make 10X TMRE Labeling Solution. 2 μ M is used in this protocol; 0.1 to 10 μ M is recommended based on different cell lines.
4. If CCCP is used as positive control, allow CCCP solution to equilibrate to room temperature before use.

A. Protocol for suspension cells

1. Suspend cells in warm medium 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 μ l of 50 mM CCCP (supplied with this kit) to the control tube for a 50 μ M final concentration; incubate cells at 37° C for 15 min.
3. Add 100 μ l of the 2 μ M TMRE Labeling Solution to each sample (200 nM final concentration) and incubate cells in the incubator (37° C and 5% CO_2) for 15 to 30 min.
Note: 200 nM TMRE is recommended in this protocol. For best results, a titration of TMRE is recommended.
4. Centrifuge sample at 300 g for 5 min, then remove the supernatant.
5. Wash cells once with 1 ml warm 1X PBS wash buffer, repeat step 4.
6. Resuspend cells into 1000 μ l warm 1X PBS.
7. Analyze sample on a flow cytometer. If samples are analyzed on plate reader, transfer 100 μ l/ cell suspension/well to a black 96-well plate with a clear bottom and read plate on the plate reader. The settings are: excitation about 550 nm and emission about 580 nm.

B. Protocol for adherent cells

1. Plate cells to a 96-well plate in warm culture medium and culture cells in incubator overnight to allow cells to attach to the plate. A typical cell number is between $1-5 \times 10^4$ cells/ well. A cell number titration may be necessary for optimal results.
2. Aspirate the medium from the plate and add test compounds in growth medium or 1X PBS to plate at 100 μ l/well at desired concentration and incubate cells for desired time. Compound titration and incubation time course can help to determine the best assay conditions. For positive control (mitochondrial membrane potential loss), add CCCP (supplied with this kit) to the control wells for a 50 μ M final concentration and incubate cells at 37° C for 15 min. For example, add 1 μ l of 50 mM stock CCCP to 100 μ l medium to make 500 μ M CCCP; then add 10 μ l of this 500 μ M CCCP to each well containing 100 μ l medium to get final concentration of 50 μ M.
3. Add 10 μ l of 2 μ M TMRE Labeling Solution to each well to get a final concentration of 200 nM and place plate in an incubator (37° C and 5% CO_2) for 20 min.
Note: 200 nM TMRE is recommended in this protocol. For best results, a titration of TMRE is recommended.
4. Aspirate the solution from the plate.
5. Wash plate 3 times with warm 1X PBS and then add 100 μ l/well 1X PBS to the plate.
6. Analyze samples on the plate reader. The settings are: excitation about 550 nm and emission about 580 nm.



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, 9808F, 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
 Website www.cellsignal.com
 Email address support@cellsignal.com
 Company phone number 978-867-2300
 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

Hazards not otherwise classified (HNOC) None.

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
 Odor No information available
 Color Colorless
 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.

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.

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.**

Product does not present an aquatic toxicity hazard based on known or supplied information.

Chemical Name	Toxicity to algae	Toxicity to fish	Toxicity to daphnia and other aquatic invertebrates
sodium chloride	-	LC50 4747 - 7824 mg/L (Oncorhynchus mykiss) 96 h LC50 12946 mg/L (Lepomis macrochirus) 96 h LC50 5560 - 6080 mg/L (Lepomis macrochirus) 96 h LC50 5420 - 6700 mg/L (Pimephales promelas) 96 h LC50 7050 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

Page 5 / 7

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

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

Page 7 / 7

North American Inventory Listing.

Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL
sodium chloride	Listed	Not Listed	Listed	Not Listed

Canadian Workplace Hazardous Materials Information System (WHMIS) Classification.

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

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.

Page 6 / 7



SAFETY DATA SHEET (SDS): According to the OSHA Hazard Communication Standard 29 CFR 1910.1200
Issuing Date: 2014-02-26 Revision Date: 2014-02-27 Version: 1

SECTION 1. Identification**Product identifier**

Product No.	13472
Product name	TMRE
Other means of identification	13472M, 13472S

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 TEL: +1 978 867 2300 FAX: +1 978 867 2400 www.cellsignal.com
Website	support@cellsignal.com
Email address	978-867-2300
Company phone number	In case of emergency call CHEMTREC 1-800-424-9300
Emergency telephone number	

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

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

Acute toxicity - Oral	Category 4
Acute toxicity - Inhalation (Dusts/Mists)	Category 4
Skin corrosion/irritation	Category 2
Serious eye damage/eye irritation	Category 2

GHS Label elements, including precautionary statements

Signal Word

Page 1 / 7

Warning**Hazard statement(s)**

Harmful if swallowed
Harmful if inhaled
Causes skin irritation
Causes serious eye irritation

Precautionary Statement(s)

Wear protective gloves/protective clothing/eye protection/face protection
IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
Rinse mouth
IF ON SKIN: Wash with plenty of soap and water
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

Supplementary Hazard Information

Hazards not otherwise classified (HNOC) None
Unknown Acute Toxicity 100% of the mixture consists of ingredient(s) of unknown toxicity.

SECTION 3. Composition/information on ingredients

Formula C₈H₁₂ClN₂O₂
Molecular Weight 514.95
Chemical nature Monoconstituent substance.
Synonyms TMRE; RB3096; SID 164216713

Chemical Name	CAS No.	Weight %
tetramethylrhodamine ethyl ester perchlorate	115532-52-0	100

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 Rinse mouth.

Most important symptoms and effects, both acute and delayed

No information or data specific to the product on this toxicological (health) effect is available.

Indication of any immediate medical attention and special treatment needed

Notes to physician 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

Do not flush into surface water or sanitary sewer system.

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 Use personal protective equipment. Cover powder spill with plastic sheet or tarp to minimize spreading and keep powder dry. Take up mechanically and collect in suitable container for disposal. Avoid dust formation. Clean contaminated surface thoroughly.

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 Do not eat, drink or smoke when using this product.

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

Physical state Solid
Appearance Lyophilized
Odor No information available
Color Red
Odor Threshold No information available
pH No information available
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
Molecular Weight 514.95
VOC content No information available
Viscosity No information available
Density 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

May emit toxic fumes under fire conditions - carbon monoxide, carbon dioxide, nitrogen oxides, and hydrogen chloride.

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

Inhalation Harmful by inhalation.
Eye contact Expected to be an irritant based on components.
Skin contact Expected to be an irritant based on components.
Ingestion Harmful if swallowed.

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.

Unknown Acute Toxicity 100% of the mixture consists of ingredient(s) of unknown toxicity.

ATEmix (oral) 500 mg/kg

ATEmix (inhalation-dust/mist) 1.5 mg/l

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

Symptoms No information available.
Corrosivity 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.
Developmental Toxicity No information available.
Teratogenicity 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

100% of the mixture consists of components(s) of unknown hazards to the aquatic environment

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

All of the components in the product are on the following inventory lists:

TSCA	-
DSL	-
NDSL	Complies

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

SAFETY DATA SHEET (SDS): According to the OSHA Hazard Communication Standard 29 CFR 1910.1200
Issuing Date: 2014-02-19 Revision Date: 2014-02-28 Version: 1

SECTION 1. Identification**Product identifier**

Product No.	13550
Product name	CCCP
Other means of identification	13550M, 13550S, MTL4661L

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 TEL: +1 978 867 2300 FAX: +1 978 867 2400 www.cellsignal.com
Website	www.cellsignal.com
Email address	support@cellsignal.com
Company phone number	978-867-2300
Emergency telephone number	In case of emergency call CHEMTREC 1-800-424-9300

SECTION 2. Hazard(s) identification

This chemical 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	Category 4

GHS Label elements, including precautionary statements

Signal Word
Warning

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**U.S. EPA Label Information**

This product does not contain any substances regulated as pesticides.

SECTION 16. Other information

Issuing Date: 2014-02-26

Revision Date: 2014-02-27

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

Hazard statement(s)

Causes skin irritation
Causes serious eye irritation
Combustible liquid

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

Hazards not otherwise classified (HNOC) None

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 or data specific to the product on this toxicological (health) effect is available.

Indication of any immediate medical attention and special treatment needed

Notes to physician 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

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.

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

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. Vapors may form explosive mixtures with air. Incompatible materials.

Hazardous polymerization 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 555-60-2	= 100 mg/kg (Rat)	= 300 mg/kg (Rat)	= 0.5 mg/l (Rat) Dust/mist
Dimethyl sulfoxide 67-68-5	= 14500 mg/kg (Rat)	= 40 g/kg (Rat)	-

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

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	ACGIH TLV	OSHA PEL	NIOSH REL
[[3-chlorophenyl]hydrazono]malononitrile 555-60-2	-	TWA: 5 mg/m ³ (vacated) TWA: 5 mg/m ³ S	IDLH: 25 mg/m ³ CN

(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
Odor Sulphurous
Color Yellow
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/water log Pow: -2.03
Autoignition temperature No information available

Symptoms No information available.
Corrosivity 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.
Developmental Toxicity No information available.
Teratogenicity 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.
Endocrine Disruptor Information [[3-chlorophenyl]hydrazono]malononitrile is a suspected endocrine disruptor

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 67-68-5	EC50 12350 - 25500 mg/L (Skeletonekema 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

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

Chemical Name	Octanol-Water Partition Coefficient
Dimethyl sulfoxide 67-68-5	-2.03

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

All of the components in the product are on the following inventory lists:

TSCA	Complies
DSL	-
NDSL	-

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	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 - 555-60-2	-	Listed	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

Chemical Name	New Jersey	Massachusetts	Pennsylvania
[(3-chlorophenyl)hydrazono]malononitrile - 555-60-2	Listed	-	Listed

Dimethyl sulfoxide 67-68-5	Listed	-	-
----------------------------	--------	---	---

U.S. EPA Label Information

This product does not contain any substances regulated as pesticides.

SECTION 16. Other information

Issuing Date: 2014-02-19
Revision Date: 2014-02-28

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