Protease/Phosphatase Inhibitor Cocktail (100X)

1 ml

#5872

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

Description: When diluted in lysis buffer to a final concentration of 1X, the Protease/Phosphatase Inhibitor Cocktail prevents protein degradation and phosphatase by endogenous proteases and phosphatases present in the whole cell extract. The 100X cocktail is a clear light yellow to light green liquid.

Background: Dynamic protein phosphorylation is a key cellular signaling mechanism by which a broad spectrum of cellular processes is regulated. In order to study the phosphorylation status of specific target proteins, the phosphorylated residue of interest must remain intact. When cells are lysed to make whole cell extracts, a loss of normal cellular signaling regulation occurs, and phosphatases within the cell extract are free to dephosphorylate proteins in an uncontrolled manner. The addition of phosphatase inhibitors to the cell lysis buffer aids in the preservation of phosphorylated residues at the time of cell disruption.

This same loss of normal cellular control when generating whole cell extracts also leads to uncontrolled degradation of proteins by endogenous proteases. The addition of protease inhibitors to the cell lysis buffer aids in the preservation of target proteins in the cell extract.

Directions for Use:
1. Briefly vortex the Protease/Phosphatase Inhibitor Cocktail (100X) before use.
2. Just prior to lysing cells, dilute the cocktail 1:100 in desired lysis buffer to obtain a 1X working concentration.

Solutions and Reagents: The Protease/Phosphatase Inhibitor Cocktail (100X) is composed of a proprietary mix of Aprotinin, Bestatin, E64, and Leupeptin to promote broad spectrum protection against endogenous proteases and sodium fluoride, sodium pyrophosphate, β-glycerophosphate, and sodium orthovanadate to promote broad spectrum protection against endogenous serine/threonine and tyrosine phosphatases. The cocktail does not contain EDTA (a metalloprotease inhibitor) which can be incompatible with some downstream applications (i.e. protein assays, 2D electrophoresis, etc.). If EDTA is desired as a protease inhibitor it can be added to the cell lysis buffer at a final working concentration of 5mM.

Western blot analysis of extracts from NIH/3T3 cells, serum-starved overnight and treated with hPDGF-BB #8912 (100ng/ml, 5min), prepared in lysis buffer in the absence of phosphatase inhibitors (left) or with Protease/Phosphatase Inhibitor Cocktail (100X) #5872 added (right), and incubated at 37°C for the indicated time points, using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (upper) or Akt (pan) (C267E7) Rabbit mAb #4691 (lower). In the absence of phosphatase inhibitors, the phospho-Akt signal is preserved through all time points monitored.

Western blot analysis of extracts from NIH/3T3 cells, prepared in lysis buffer in the absence of protease inhibitors (left) or with Protease/Phosphatase Inhibitor Cocktail (100X) #5872 added (right), and incubated at 37°C for the indicated time points, using β-Catenin (D10A8) XP® Rabbit mAb #8480. In the absence of protease inhibitors, β-Catenin signal fades within 3 hr after harvest, indicating protein degradation. In the presence of the protease inhibitor cocktail, the β-Catenin degradation is slowed significantly and signal is still present at 20 hr following harvest.

Storage: Store the undiluted 100X cocktail at 4°C. Do not freeze. This product is stable for 12 months.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

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

Product Name: Protease/Phosphatase Inhibitor Cocktail (100X)
Product Number: 5872
Identified Uses: For Research Use Only (RUC). Not intended for use in humans or animals. Not intended for therapeutic or diagnostic procedures.
Manufacturer/Supplier: Cell Signaling Technology, Inc.
3 Treas Lane
Danvers, MA 01923 USA
Phone #: 1-978-887-2300
Emergency Tel: 1-978-576-6737

2. HAZARDS IDENTIFICATION

Emergency Overview
Warning! This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Physical State: Liquid

Routes of Entry:
- Physical State: Contains material that can cause target organ damage.
- Suspect cancer hazard – contains material which may cause cancer.

Physical State: Liquid

Potential Health Effects

Acute Toxicity
- Eyes: Irritating to eyes.
- Skin: Harmful if contact with skin
- Inhalation: Tonic if inhaled, irritating to respiratory system.
- Ingestion: Tonic if swallowed

Chronic Effects
- Carcinogenic effects: Contains material which may cause cancer. Risk of cancer depends on duration and level of exposure.
- Mutagenic effects: No known significant effects or critical hazards.
- Teratogenic effects: No known significant effects or critical hazards.
- Reproductive toxicity: No information available.

Target organ effects: Contains material which causes damage to the following organs: Heart, Teeth. Contains material which causes damage to the following organs: Kidneys, gastrointestinal tract, upper respiratory tract, skin, bones, central nervous system (CNS), eye, lens, or cornea.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Table 3: Ingredients and their Hazard Classifications

<table>
<thead>
<tr>
<th>Ingredient Name</th>
<th>CAS No.</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disodium beta-glycerophosphate</td>
<td>87-28-9</td>
<td>3-50%</td>
</tr>
<tr>
<td>Disodium ethylenediaminetetraacetic acid</td>
<td>7552-88-1</td>
<td>1-3%</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>7681-90-4</td>
<td>1-3%</td>
</tr>
<tr>
<td>Trisodium tetraoxovanadate</td>
<td>13721-39-6</td>
<td>1-3%</td>
</tr>
<tr>
<td>Tryptophan, pancreatic basic</td>
<td>9007-70-1</td>
<td>0.1-1%</td>
</tr>
</tbody>
</table>

4. FIRST AID MEASURES

Eye Contact: Rinse immediately with plenty of water. Get medical attention.
Skin Contact: Rinse immediately with soap and plenty of water. Get medical attention.
Inhalation: Move to fresh air. Get medical attention.
Ingestion: Call Poison Control Center immediately. Never give anything by mouth to an unconscious person. Rinse mouth with water.

Notes to Physician: Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flash Point: See Section 9. Physical and Chemical Properties

Suitable Extinguishing Media: Use dry chemical

Special exposure hazards: Not available

Special protection for fire fighters: Wear appropriate self-contained breathing apparatus and protective unit

Other information: No data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions: Do not touch or walk though spilled material. Wear personal protective equipment.

Environmental precautions: Do not let product enter drains.

Clean up methods: Keep in suitable closed containers for disposal.

Other information: See sections 12 and 13 for additional information.

7. HANDLING AND STORAGE

Safe handling advice: Avoid contact with eyes and skin. Ensure adequate ventilation.

Storage conditions: Keep container tightly closed in a cool dry location.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure Limits: Ingredients with workplace control parameters

9. PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical State</td>
<td>Liquid</td>
</tr>
<tr>
<td>Appearance</td>
<td>Odorless</td>
</tr>
<tr>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td>pH</td>
<td>Not available</td>
</tr>
<tr>
<td>Water Solubility</td>
<td>Soluble</td>
</tr>
<tr>
<td>Melting point/freezing point</td>
<td>Not available</td>
</tr>
<tr>
<td>Boiling point</td>
<td>Not available</td>
</tr>
<tr>
<td>Evaporation rate</td>
<td>Not available</td>
</tr>
<tr>
<td>Flash point</td>
<td>Not available</td>
</tr>
<tr>
<td>Autoignition temperature</td>
<td>Not available</td>
</tr>
<tr>
<td>Flammability limits in air</td>
<td>Not available</td>
</tr>
<tr>
<td>Explosive properties</td>
<td>Not available</td>
</tr>
<tr>
<td>VOC content</td>
<td>No data available</td>
</tr>
<tr>
<td>Decomposition temperature</td>
<td>Not available</td>
</tr>
</tbody>
</table>

10. STABILITY AND REACTIVITY

Stability: Stable under normal conditions

Conditions/Materials to avoid: No data available

Hazardous decomposition products: No data available

11. TOXICOLOGICAL INFORMATION

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

Routes of Exposure

Potential Health Effects

12. ECOLOGICAL INFORMATION

Ecotoxicity: The environmental impact of this product has not been fully investigated. No known significant effects or critical hazards.

Persistence and degradability: Not available

Bioaccumulation: Not available

Mobility: Not available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods: Dispose of in accordance with all applicable environmental laws and regulations.

14. TRANSPORT INFORMATION

IATA: Not regulated as dangerous goods
DOT: Not regulated as dangerous goods

Cell Signaling Technology, Inc
www.cellsignal.com

Material Safety Data Sheet (MSDS)
Revision Date 2012-06-04
Version 2

Material Safety Data Sheet (MSDS)
Revision Date 2012-06-04
Version 3
MEX
Not regulated as dangerous goods

15. REGULATORY INFORMATION

HCS Classification
Toxic material
Initiating material
Carcinogen
Target Organ Effects

International Inventories
TSCA (8a) PARR - Trisodium tetraoxovanadate
EINECS/ELINCS -
INCI -
KECL -
PICS -
AICS -
NZIoC -

U.S. Federal Regulations
SARA 302/304/311/312 extremely hazardous products: No products were found.
SARA 302/304/311/312 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: Sodium fluoride. Trisodium tetraoxovanadate
SARA 311/312 MSDS Distribution:
Sodium fluoride: Immediate (acute) health hazard, Delayed (chronic) health hazard.
Trisodium tetraoxovanadate: Delayed (chronic) health hazard.
Commerce Control List precursor: Sodium fluoride.

Clean Water Act
This product contains the following components are listed as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42): Sodium fluoride.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contain any substances regulated as hazardous air pollutants (HAPs) under Section 112 of the Clean Air Act Amendments of 1990.

U.S. State Regulations
Massachusetts: The following components are listed: Sodium fluoride.
New York: The following components are listed: Sodium fluoride.
New Jersey: The following components are listed: Sodium fluoride.
Pennsylvania: The following components are listed: Sodium fluoride.
U.S. Inventory (TSCA 8b): All least one component is listed.

Canada
WHMIS Hazard Class
D1B Toxic. Material causing immediate and serious toxic effects.
D2A Very toxic. Materials causing other toxic effects.
D2B Toxic. Materials causing other toxic effects.

Canadian NPRI
The following components are listed: Sodium fluoride, Vanadium.

CEPA Toxic Substances
The following components are listed: Inorganic fluorides.

Canada Inventory
At least one component is listed.

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR.

16. OTHER INFORMATION

Revision date 2012-05-22
Revision Note ***Indicates updated section

Disclaimer
The information provided on this material safety data sheet is to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as 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 material or in any process, unless specified in the text.