Cell Fractionation Kit

#9038 Store at -20°C

1 Kit (20 assays)

Description:
The Cell Fractionation Kit is designed to provide a fast and efficient way of separating cultured cells into three distinct fractions: cytoplasmic, membrane/organelle, and nuclear/cytoskeletal. These fractions can then be analyzed by SDS-PAGE and western blotting. The kit includes enough buffer for 20 assays.

Background:
Cellular fractionation allows for the extraction of cellular proteins into distinct compartments. This is achieved by the use of detergents that take advantage of the inherent qualities and composition of different cellular membranes (1). Cellular fractionation has been important for defining the localization of many proteins, observing the translocation of proteins, and determining protein-protein complexes, such as cytoskeletal-associated proteins (2,3). Thus, detergent-based cellular fractionation separates cellular components with greater ease and speed compared to a more laborious density centrifugation method (4).

Storage: Upon receipt, Protease Inhibitor Cocktail 5871 should be stored at 4°C. All other components should be stored at -20°C.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Products Included

<table>
<thead>
<tr>
<th>Product</th>
<th>Product #</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic Isolation Buffer (CIB)</td>
<td>9041</td>
<td>1 x 10 ml</td>
</tr>
<tr>
<td>Membrane Isolation Buffer (MIB)</td>
<td>9048</td>
<td>1 x 10 ml</td>
</tr>
<tr>
<td>Cytoskeletal/Nuclear Isolation Buffer (CyNIB)</td>
<td>9049</td>
<td>1 x 5 ml</td>
</tr>
<tr>
<td>Protease Inhibitor Cocktail (100X)</td>
<td>5871</td>
<td>1 x 250 µl</td>
</tr>
</tbody>
</table>

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Specificity/Sensitivity: This kit is intended to be used with cultured cell lines that are either adherent or in suspension. The Cell Fractionation Kit allows for the determination of the subcellular localization of proteins through the separation into three distinct fractions.

“Efficiency of separation can be measured using Cell Signaling Technology’s Cell Fractionation Antibody Sampler Kit #11843.”

Background References:

Western blot analysis of cell fractions from HeLa cells using MEK1/2 (D1A5) Rabbit mAb #8727, AIF (D3902) XP® Rabbit mAb #5318, Histone H3 (D1H2) XP® Rabbit mAb #4499, and Vimentin (D21H3) XP® Rabbit mAb #5741 (as part of the Cell Fractionation Antibody Sampler Kit #11843) showing cytoplasmic, organelle/membrane, and nuclear/cytoskeletal localization. Whole cell lysates (WCL) were used to represent total protein. Cytoplasmic proteins (Cyto) were isolated using CIB buffer. Integral membrane and organelle proteins (Mem) were isolated using MIB buffer. Nuclear and cytoskeletal proteins (Nuc) were isolated using CyNIB buffer.
Cell Fractionation Protocol

A Buffers

- **Cytoplasm Isolation Buffer (CIB)** – 10 ml, Store at -20°C.
- **Membrane Isolation Buffer (MIB)** – 10 ml, Store at -20°C.
- **Cytoskeleton/Nucleus Isolation Buffer (CyNIB)** – 5 ml, Store at -20°C.
- **Protease Inhibitor Cocktail (100X) (#5871)** – 250 µl, Store at 4°C.

B Notes

- All steps, except for the addition and sonication of the CyNIB buffer, should be done on ice or at 4°C.
- Adherent or suspension cultured cells can be used for this assay.
- 1X protease inhibitors (#5871) (5 µl of 100X per 500 µl buffer, included in kit) and 1 mM fresh PMSF (#8553) (2.5 µl of 200 mM PMSF per 500 µl buffer, not included in kit) should be added to each buffer immediately before use.
- Phosphatase inhibitors are already included in the buffers. There is no need to add them.
- Please refer to Table 1 for the appropriate volumes for your specific cell concentration.
- The volumes given in Sections D and E are based on cell counts obtained from HeLa cells at ~90% confluency in a 10 cm cell culture dish (5x10^6 cells).
- If the CyNIB buffer is cloudy after thawing, please warm the solution in a 37°C water bath until the solution is clear.
- Be cautious when saving fractions so that you do not get any contamination from the resulting pellet.
- All lysates should be stored at -20°C for short term storage (less than 1 month) or -80°C for long term storage (greater than 1 month).

C Isolating Cell Population

For adherent cells
1. Wash plate with cold 1X PBS.
2. Trypsinize the plate.
3. Add cold growth media to deactivate trypsin.

For both adherent and suspension cells
1. Spin down cells at 350 x g for 5 min.
2. Aspirate media.
3. Wash cell pellet with cold 1X PBS.
4. Resuspend pellet in 0.5 ml of cold 1X PBS.
5. Count live cells using Trypan Blue and a hemacytometer.

D Detection of Proteins

1. Aliquot 100 µl of cell suspension into a 1.5 ml tube for the whole cell lysate (WCL).
2. Add 60 µl of 3X SDS Loading Buffer with DTT (#7722) to make a final volume of 160 µl of WCL.
3. Sonicate WCL tube for 15 sec at 20% power 3 times, heat for 5 min at 95°C, and centrifuge for 3 min at 15,000 x g.

E Cell Fractionation

1. Aliquot the remaining 400 µl into a 1.5 ml tube.
2. Centrifuge for 5 min at 500 x g at 4°C.
3. Aspirate the supernatant.
4. Resuspend pellet in 500 µl of CIB.
5. Vortex for 5 sec.
6. Incubate on ice for 5 min.
7. Centrifuge for 5 min at 500 x g.
8. Save the supernatant. This is the **Cytoplasmic Fraction**.
9. Resuspend pellet in 500 µl of MIB.
10. Vortex for 15 sec.
11. Incubate on ice for 5 min.
12. Centrifuge for 5 min at 8,000 x g.
13. Save the supernatant. This is the **Membrane and Organelle Fraction**.
14. Resuspend pellet in 250 µl of CyNIB.
15. Sonicate for 5 sec at 20% power 3 times. This is the **Cytoskeletal and Nuclear Fraction**.

F Western Blot

1. Add 60 µl of 3X SDS Loading Buffer with DTT (#7722) for every 100 µl of supernatant.
2. Boil each sample for 5 min at 95°C and centrifuge for 3 min at 15,000 x g.
3. Load 15 µl of each fraction along with 15 µl of WCL.

Table 1: Volumes in µl for WCL or buffer at indicated cell numbers.

<table>
<thead>
<tr>
<th>Cell Count</th>
<th>2.5 x 10^6 cells</th>
<th>5 x 10^6 cells</th>
<th>7.5 x 10^6 cells</th>
<th>1 x 10^7 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCL</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>CIB</td>
<td>250</td>
<td>500</td>
<td>750</td>
<td>1000</td>
</tr>
<tr>
<td>MIB</td>
<td>250</td>
<td>500</td>
<td>750</td>
<td>1000</td>
</tr>
<tr>
<td>CyNIB</td>
<td>125</td>
<td>250</td>
<td>375</td>
<td>500</td>
</tr>
</tbody>
</table>
1. PRODUCT AND COMPANY IDENTIFICATION

Product Code(s): 9041S
Product Name: Cytosolic Isolation Buffer (CIB)

2. HAZARDS IDENTIFICATION

The product does not contain any substances which at their given concentration, are considered to be hazardous to health.

3. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS-No</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoride</td>
<td>7681-49-4</td>
<td>95</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50-05-8</td>
<td>95</td>
</tr>
</tbody>
</table>

4. FIRST AID MEASURES

Eye contact: Rinse immediately with plenty of water and seek medical advice.
Skin contact: Rinse immediately with plenty of water and seek medical advice.
Inhalation: Move to fresh air.

5. FIRE-FIGHTING MEASURES

Flammable Properties: Not flammable.
Suitable Extinguishing Media: Water spray.
Protective Equipment and Precautions for Firefighters: As in any fire, wear self-contained breathing apparatus pressure-demand, NIOSH/MSHA approved or equivalent and full protective gear.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions: Avoid contact with skin, eyes and clothing.
Methods for Containment: Prevent further leakage or spillage. If safe to do so:
Methods for cleaning up: Prevent product from entering drains.

7. HANDLING AND STORAGE

Advice on safe handling: Ensure adequate ventilation. Handle in accordance with good industrial hygiene and safety practice.
Technical measures/Storage conditions: Keep container tightly closed. Recommended storage temperature -20°C.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION


9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State: Liquid
Appearance: Clear
Color: None
Odor: None
pH: 7.5
Melting point/range: No information available
Boiling Point/Range: No information available
Flammability limits in air: No information available
Explosive properties: Not applicable
Autoignition temperature: No information available
Flash point: No information available
Explosive limits in air: No information available
Initial flash point: No information available
Decomposition temperature: No information available

10. STABILITY AND REACTIVITY

Stability: Stable under normal conditions.
Conditions to Avoid: Strong acids, oxidizing agents.

11. TOXICOLOGICAL INFORMATION

Carcinogenicity: None of the component present at levels greater than or equal to 0.1% is identifiable as probable or confirmed carcinogen by IARC, ACGIH, NTP, or OSHA.

12. ECOLOGICAL INFORMATION

N/A

Cell Signaling Technology, Inc.
www.cellsignal.com
### 1. PRODUCT AND COMPANY IDENTIFICATION

**Product Code(s):** 9046S5  
**Product Name:** Membrane Isolation Buffer (MIB)  
**Pure substance/preparation:** Preparation  
**Identified uses:** For research use only. Not for use in humans.  
**Manufacturer:** Cell Signaling Technology, Inc.  
**Company Address:** 3 Trask Lane, Danvers, MA 01923  
**Toll Free:** 978-867-2300  
**Emergency telephone:** In case of emergency call CHEMTREC 1-800-424-9300  
**WSHES Hazard Class:** Not Determined

### 2. HAZARDS IDENTIFICATION

**Appearance Color:**  
**Odor:**  
**Potential Health Effects:**  
**Inhalation:** No known effect based on information supplied.  
**Ingestion:** No known effect based on information supplied.  
**Skin:** No known effect based on information supplied.  
**Eye:** May cause slight irritation.  
**Chronic Effects:** None known.  
**Acute Toxicity:** No known effect based on information supplied.  
**Chronic Toxicity:** No known effect based on information supplied.  
**Aggravated Medical Conditions:** None known.  
**Environmental hazard:** See Section 12 for additional ecological information.

### 15. REGULATORY INFORMATION

#### U.S. Federal Regulations

- **TSCA** - X  
- **AICS** - X  
- **PICCS** - X  
- **IECSC** - X  
- **ENCS** - X  
- **EINECS/ELINCS** - X

#### International Regulations

- **OSHA Regulatory Status**  
- **International Inventories**  
- **U.S. - TSCA (Toxic Substances Control Act) - Section 8(a)**  
- **Chemical Name**  
- **Compounds Listed**

### 3. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS-No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoride</td>
<td>7681-49-4 (0.1 - 1)</td>
</tr>
</tbody>
</table>

### 4. FIRST AID MEASURES

**Eye contact:** Rinse immediately with plenty of water and seek medical advice.  
**Skin contact:** Rinse immediately with plenty of water and seek medical advice.  
**Inhalation:** Move to fresh air.
5. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid contact with skin, eyes and clothing.

Methods for Containment
Prevent further leakage or spillage if safe to do so.

Methods for cleaning up
Prevent product from entering drains.

6. HANDLING AND STORAGE

Advice on safe handling
Ensure adequate ventilation. Handle in accordance with good industrial hygiene and safety practice.

Technical measures/Storage conditions
Keep container tightly closed. Recommended storage temperature -20 °C.

7. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Measures
Showers, eyewash stations, and ventilation systems.

Hygiene measures
When using, do not eat, drink or smoke. Wear suitable gloves and eye/face protection. Wash hands with water as a precaution. Regular cleaning of equipment, work area and clothing is recommended. Avoid breathing vapors, mist or gas.

Personal Protective Equipment

8. PHYSICAL AND CHEMICAL PROPERTIES

Chemical Name
Sodium fluoride
Chemical State
Solid
Boiling Point
238 °C
Melting Point
307-309 °C
Evaporation Rate
No information available.
Initial Boiling Point
No information available.
Vapour Pressure
No information available.
Explosive properties
No information available.
Flammability
Not flammable.
Autoignition temperature
No information available.
Flammability Limits in Air
No information available.
Explosion properties
No information available.
Flash Point
No information available.
Decomposition Temperature
No information available.

9. TOXICOLOGICAL INFORMATION

Acute Health Hazard
No
Chronic Health Hazard
None under normal use.

Target Organ Effects
None known.

10. STABILITY AND REACTIVITY

Stability
Stable under normal conditions.

Conditions to Avoid
Strong acids, oxidizing agents.

Hazardous Decomposition Products
None under normal use.

11. ECOLOGICAL INFORMATION

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

12. TRANSPORT INFORMATION

DOT
Not regulated.
MEX
Not regulated.
IATA
Not regulated.

13. REGULATORY INFORMATION

OSHA Regulatory Status
This product is not regulated by the Occupational Safety and Health Administration (OSHA).

Controlled Code Status
This product is not regulated by the Environmental Protection Agency (EPA).

14. OTHER INFORMATION

Revision Date:
2012-11-08

References
For more information, please visit the manufacturer’s website (www.cellsignal.com) or contact the manufacturer directly.
1. PRODUCT AND COMPANY IDENTIFICATION

Product Code(s): 92495
Product Name: Cytokine/Hormone Isolation Buffer

2. HAZARDS IDENTIFICATION

Emergency Overview
WARNING
Intake, Ingestion
Inhalation
Skin contact
Eye contact
Skin HYPOSENSITIZATION
Pre-existing eye disorders. Respiratory disorders
Chronic toxicity
Chronic effects

Waste Hazard
Class:
Physical State
Odor

OSHA Regulatory Status
The material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). Irritant effect on eyes.

Potential Health Effects
Acute Toxicity
Skin
Inhalation

3. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS-No</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>57-50-1</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>91-02-0</td>
<td>0 - 1</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>151-21-3</td>
<td>0.2 - 1.2</td>
</tr>
</tbody>
</table>

4. FIRST AID MEASURES

Eye contact
Rinse immediately with plenty of water and seek medical advice.

Skin contact
Rinse immediately with plenty of water and seek medical advice.

Inhalation
Move to fresh air.

5. FIRE-FIGHTING MEASURES

Fire-fighting Media
Suitable: Water. Inert gas or foam. Avoid metal containing fire extinguishing agents.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid contact with skin, eyes and clothing.

Methods for Containment
Remove entry sources. Prevent further leakage or spillage if safe to do so.

7. HANDLING AND STORAGE

Advice on safe handling
Ensure good ventilation. Do not eat, drink or smoke when handling. Use appropriate protective equipment.

Technical measures/Storage conditions
Keep container tightly closed. Recommended storage temperature: -20 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Measures
Showers, eyewash stations, and ventilation systems.

5. HAZARDS IDENTIFICATION

NFPA
Health Hazard: 0
Flammability: 0
Stability: 0
Physical and chemical hazards:

NO information available.

6. ACCIDENTAL RELEASE MEASURES

Prevent further leakage or spillage if safe to do so.

7. HANDLING AND STORAGE

Ensure adequate ventilation. Handle in accordance with good industrial hygiene and safety practice.

Technical measures/Storage conditions
Keep container tightly closed. Recommended storage temperature: -20 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure Guidelines
This product does not contain any hazardous materials with occupational exposure limits established by the regional regulatory bodies.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State
Liquid
Appearance
Clear
Color
Colorless
pH
pH = 7.5
Solubility
Soluble in water
Melting point/range
No information available
Boiling point/Range
No information available
Flammable Properties
Not flammable.
Flash point
No information available
Flammable Limits in Air
Not flammable.
Inflammability Limits In Air
Not flammable.
Explosion hazards
No information available
VOC Content
No information available
Initial Boiling Point
No information available
Evaporation Rate
No information available
Decomposition Temperature °C
No information available

10. STABILITY AND REACTIVITY

Stability
Stable under normal conditions.

11. TOXICOLOGICAL INFORMATION

Carcinogenicity
No component of this product present at levels greater than or equal to 0.1% is identical as probable, possible or confirmed carcinogen by IARC, ACGIH, NTP, or OSHA.

Target Organ Effects
Eyes, Respiratory system.
12. ECOLOGICAL INFORMATION

Persistence and degradability
No information available.

Bioaccumulation
No information available.

Mobility
No information available.

13. DISPOSAL CONSIDERATIONS

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

14. TRANSPORT INFORMATION

15. REGULATORY INFORMATION

OSHA Regulatory Information
This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). Irritant effect on eyes.

International inventories
TSCA -
OSHA -
EMC -
ECB -
KECL -
PICCS -
ENCS -

U.S. Federal Regulations
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.

TSCA
Chemical Name U.S. - TSCA (Toxic Substances Control Act) - Section 8(a) - Chemical-Specific Reporting and Recordkeeping
Component CBRA - Reportable Quantities CBRA - Toxic Pollutants CBRA - Priority Pollutants CBRA - Hazardous Substances
Sodium fluoride 360-464-1 (0.1 - 1)

SARA 311/312 Hazard Categories
Acute Health Hazard No
Chronic Health Hazard No
Fire Hazard No
Sudden Release of Pressure Hazard No
Reactive Hazard No

16. OTHER INFORMATION

Revision Date: 0011-08-20
Revision Note: No information available.

Disclaimer
The information provided on this MSDS 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 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 test.

End of Material Safety Data Sheet
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
Water

Special Exposure Hazards
No data 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 through spilled material. Wear personal protective equipment

Environmental Precautions
Do not let product enter drains.

Clean Up Methods
Soak up with absorbent material. 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.

Incompatibilities
No data available

Specific End Uses
No data available

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure Limits
Ingredients with workplace control parameters

Respiratory Protection
In case of insufficient ventilation wear suitable respiratory equipment.

Eye Protection
Safety glasses with side shields

Hand Protection
Compatible chemical resistant gloves.

9. PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties

Physical State
liquid

Appearance
clear

Odor
odorless

pH
no data available

Solubility
Soluble

Melt point/freezing point
no data available

Boiling Point
no data available

Evaporation Rate
no data available

Flash Point
Closed cup >100°C (>212’F) [Product does not sustain combustion.]

Autoignition Temperature
no data available

Flammability Limits in air
no data available

Explosive Properties
no data available

VOC content
no data available

Decomposition Temperature
no data available

10. STABILITY AND REACTIVITY

Stability
Stable under normal conditions

Conditions/Materials to avoid
No data available

Hazardous Decomposition Products
Under normal conditions of storage and use, hazardous decomposition products should not be produced.

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
Eyes
Corrosive to eyes. Causes burns.

Skin
Corrosive to skin. Causes burns. Harmful in contact with skin.

Inhalation
Corrosive to respiratory system. Exposure to decomposition products may cause a health hazard. Serious effects may be delayed following exposure.

Ingestion
Harmful if swallowed. May cause burns to mouth, throat and stomach.

Chronic 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

MEX
Not regulated as dangerous goods

15. REGULATORY INFORMATION

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
2012-06-04
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.

End of Material Safety Data Sheet