

FCM Permeabilization buffer (1X): sc-3623

DESCRIPTION

The FCM permeabilization buffer (1X) can be used to permeabilize cells prior to antibody-based intracellular protein labeling for flow cytometry.

Product size: 100 ml.

APPLICATION NOTES

FIXED AND PERMEABILIZED CELLS FOR INTRACELLULAR STAINING

- Once supernatant is aspirated from cell preparation, resuspend pellet in enough 1X PBS to have a final cell concentration of 10 million cells/ml.

OPTIONAL: For mouse Fc Receptor blocking, incubate the cell suspension with 1 µg of sc-18867 L per 1 ml of cell suspension for 10 minutes.

- Block by incubating the cell suspension with 1 mg of sc-18867 L per 1 ml of cell suspension for 10 minutes.
- Resuspend pellet in approximately 50 ml 1X PBS to wash away any excess blocking antibody.
- Centrifuge for 5 minutes at 1000 RPM.
- Once supernatant is aspirated from cell preparation, resuspend pellet in FCM Fixation Buffer (sc-3622). Use 1 mL per million cells.
- Incubate for 30 minutes at room temperature on a rotator.
- Centrifuge for 5 minutes at 1500-2000 RPM. Cells get more buoyant after fixation. If pellet is too small, spin again at a higher RPM, but do not exceed 3000 RPM.
- Pour off supernatant. Cells may be lost if aspirating from this point on, so always decant. Use a quick motion and don't allow the supernatant to wash back and forth over the cells.
- Resuspend pellet in approximately 50 ml 1X PBS to wash away any excess Fixation Buffer.
- Centrifuge for 5 minutes at 1500-2000 RPM.
- Decant supernatant. At this point, cells can be resuspended in a small amount of PBS and stored for up to 1 month at 4° C. To permeabilize at this time, proceed to next step.

NOTE: You should only proceed with permeabilization if you can stain immediately afterwards.

- If cells have been stored in PBS, centrifuge for 5 minutes at 1500-2000 RPM and decant supernatant.
- Break up cell pellet and dropwise add the same amount of COLD (stored at -20° C) FCM Permeabilization Buffer, sc-3623 at 1 ml per 1 million cells. Vortex while adding.
- Incubate for 5 minutes only at RT on a rotator.
- Immediately centrifuge for 5 minutes at 2000-2500 RPM. Cells are more buoyant after permeabilization and much care must be exercised to maintain volume of cells.

NOTE: Important: If a pellet is not recovered at this step, be sure to spin again and try to recover more cells.

APPLICATION NOTES *cont.*

- Decant supernatant and add approximately 50 ml 1X PBS to wash away any excess Permeabilization Buffer.
- Centrifuge for 5 minutes at 2000-2500 RPM.
- Decant supernatant and resuspend pellet in enough FCM Wash Buffer, sc-3624, for a final cell concentration of 10 million cells/ml. In the staining steps, use FCM Wash Buffer in place of 1X PBS.

STAINING

Follow protocol for direct or indirect staining.

DIRECT STAINING

(with Fluorochrome-Conjugated Antibodies)

- Label tubes.
- Add 20 µl of fluorochrome-conjugated antibodies to tubes.
- Add 100 µl of the prepared cell suspension (equal to 1 million cells) to each tube.
- Vortex and incubate for 15-30 minutes in a covered ice bucket.
- To wash off excess antibody following staining, add 1.5-2 ml of 1X PBS to each tube.
- Centrifuge in tabletop microfuge for 5 minutes at 2000 RPM. This speed should be increased to 3000 or 4000 RPM for intracellular staining.
- Aspirate supernatant, being careful not to disturb pellet.
- Resuspend pellets in 500 µl of 1% paraformaldehyde. Tubes can be stored in the dark for 24 hours (maximum for intracellular staining) to 1 week (maximum for surface staining).

INDIRECT STAINING

(with fluorochrome-unconjugated primary antibodies and fluorochrome conjugated secondary antibodies)

- Label tubes.
- Add unconjugated primary antibodies to tubes. Use approximately 1 µg per tube.
- Add 100 µl of the prepared cell suspension (equal to 1 million cells) to each tube.
- Vortex and incubate for 15-30 min in a covered ice bucket.
- To wash off excess antibody following staining, add 1.5-2 ml of 1X PBS to each tube.
- Centrifuge in tabletop microfuge for 5 minutes at 2000 RPM (or 3000-4000 RPM for intracellular staining).
- Aspirate supernatant, being careful not to disturb pellet.
- Add 100 µl of 1X PBS to each tube. Add fluorochrome-conjugated secondary antibodies to tubes. Use 0.5-1 µg of antibody.
- Vortex and incubate for 15-30 minutes in a covered ice bucket.

APPLICATION NOTES *cont.*

- To wash off excess antibody following staining, add 1.5-2 ml of 1X PBS to each tube.
- Centrifuge in tabletop microfuge for 5 minutes at 2000 RPM (or 3000-4000 RPM for intracellular staining).
- Aspirate supernatant, being careful not to disturb pellet.
- Resuspend pellets in 500 µl of 1% paraformaldehyde. Tubes can be stored in the dark for 24 hours (maximum for intracellular staining) to 1 week (maximum for surface staining).

ACQUIRE

Acquire within 24 hours.

STORAGE

Store at room temperature.

WARNING

FCM Permeabilization buffer (1X) contains methanol; CH₃OH (CAS# 67-56-1, UN#1230, ERG #131). See MSDS for safety information.

RESEARCH USE

For research use only; not for diagnostic or therapeutic use.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. Santa Cruz Biotechnology, Inc. shall not be held liable for any damage resulting from handling or from contact with the product.

12/6/2012

MATERIAL SAFETY DATA SHEET

1 Identification of substance:





Product Name: FCM Permeabilization buffer (1X)
Catalog Number: sc-3623
Supplier: Santa Cruz Biotechnology, Inc.
 2145 Delaware Avenue
 Santa Cruz, California 95060
 800.457.3801 or 831.457.3800

Emergency: ChemWatch
 Within the US & Canada: 877-715-9305
 Outside the US & Canada: +800 2436 2255
 (1-800-CHEMCALL) or call +613 9573 3112

2 Composition/information on ingredients

- **Chemical characterization: Mixtures**
- **Description:** Mixture of the substances listed below with nonhazardous additions.

CAS NO.	Description	%
67-56-1	methanol	90%

T R23/24/25-39/23/24/25
F R11

H225
H301; H311; H331
H370

3 Hazards identification

- **Classification of the substance or mixture**



GHS02 Flame

H225 Highly flammable liquid and vapour.



GHS06 Skull and crossbones

H310 Fatal in contact with skin.

H331 Toxic if inhaled.



GHS08 Health hazard

H370 Causes damage to organs.

- **Classification according to Directive 67/548/EEC or Directive 1999/45/EC**



T; Toxic

R23/24/25-39/23/24/25: Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed.



F; Highly flammable

R11: Highly flammable.

· **Classification system:**

The classification was made according to the latest editions of international substances lists, and expanded upon from company and literature data.

· **Label elements**

· **Labelling according to EU guidelines:**

The product has been classified and marked in accordance with regulations on hazardous materials.

· **Code letter and hazard designation of product:**

T Toxic

F Highly flammable

· **Hazard-determining components of labelling:**

methanol

· **Risk phrases:**

11 Highly flammable.

23/24/25 Toxic by inhalation, in contact with skin and if swallowed.

39/23/24/25 Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed.

· **Safety phrases:**

4 Keep away from living quarters.

7/9 Keep container tightly closed and in a well-ventilated place.

16 Keep away from sources of ignition - No smoking.

36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

45 In case of accident or if you feel unwell, seek medical advice immediately.

60 This material and its container must be disposed of as hazardous waste.

· **Classification system**

· **NFPA ratings (scale 0-4)**



Health = 3

Flammability = 3

Reactivity = 0

4 First aid measures

· **General information**

Immediately remove contaminated clothing.

Symptoms of poisoning may even occur after several hours; therefore provide medical observation for at least 48 hours after the accident.

Remove breathing apparatus only after contaminated clothing have been completely removed.

In case of irregular breathing or respiratory arrest provide artificial respiration.

· **After inhalation**

Supply fresh air or oxygen; and immediately call for a doctor.

In case of unconsciousness place patient on side position for transportation.

· **After skin contact** Immediately wash with water and soap and rinse thoroughly.

· **After eye contact** Rinse opened eye for several minutes under running water.

· **After swallowing** Seek immediate medical advice.

5 Firefighting measures

· **Suitable extinguishing agents**

CO₂, ABC multipurpose dry chemical or water spray. Fight larger fires with water spray or alcohol resistant foam.

· **Special hazards arising from the substance or mixture** No further relevant information available.

· **Protective equipment:** No special measures required.

6 Accidental release measures

- **Personal precautions, protective equipment and emergency procedures** Not required.
- **Environmental precautions:** Wipe up with damp sponge or mop.
- **Methods and material for containment and cleaning up:**
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders).
Dispose contaminated material as waste according to item 13.
Ensure adequate ventilation.
- **Reference to other sections**
See Section 7 for information on safe handling.
See Section 8 for information on personal protection equipment.
See Section 13 for disposal information.

7 Handling and storage

- **Handling**
- **Precautions for safe handling**
Ensure good ventilation/exhaustion at the workplace.
Open and handle receptacle with care.
Prevent formation of aerosols.
- **Information about protection against explosions and fires:**
Keep ignition sources away - Do not smoke.
Protect against electrostatic charges.
Keep respiratory protective device available.
- **Storage**
- **Requirements to be met by storerooms and receptacles:** Store in a cool location.
- **Information about storage in one common storage facility:** Store away from oxidizing agents.
- **Further information about storage conditions:** Store in cool, dry conditions in well sealed containers.
- **Specific end use(s)** No further relevant information available.
Store at room temperature.

8 Exposure controls/personal protection

- **Additional information about design of technical systems:** No further data; see item 7.

- **Components with limit values that require monitoring at the workplace:**

67-56-1 methanol

PEL	260 mg/m ³ , 200 ppm
REL	Short-term value: 325 mg/m ³ , 250 ppm Long-term value: 260 mg/m ³ , 200 ppm
	Skin
TLV	Short-term value: 328 mg/m ³ , 250 ppm Long-term value: 262 mg/m ³ , 200 ppm
	Skin; BEI

- **Additional information:** The lists that were valid during the creation were used as basis.
- **Personal Protective Equipment**
- **General protective and hygienic measures**
Keep away from foodstuffs, beverages and feed.
Immediately remove all soiled and contaminated clothing.
Wash hands before breaks and at the end of work.
Store protective clothing separately.
Avoid contact with the eyes and skin.
- **Breathing equipment:** Not required.
- **Protection of hands:**



Chemical resistant gloves (i.e. nitrile, or equivalent).

- **Eye protection:**
Safety glasses
Tightly sealed goggles.
- **Body protection:** Protective work clothing (lab coat).

9 Physical and chemical properties

· General Information	
· Appearance:	
Form:	Liquid
· Odor:	Odorless
· Odour threshold:	Not determined.
· pH-value:	Not applicable
· Change in condition	
Melting point/Melting range:	Not determined
Boiling point/Boiling range:	64°C (147 °F)
· Flash point:	11°C (52 °F)
· Flammability (solid, gaseous)	Not applicable.
· Ignition temperature:	455°C (851 °F)
· Decomposition temperature:	Not determined.
· Auto igniting:	Product is not self igniting.
· Danger of explosion:	Product is not explosive. However, formation of explosive air/vapor mixtures are possible.
· Explosion limits:	
Lower:	5.5 Vol %
Upper:	44.0 Vol %
· Vapor pressure at 20°C (68 °F):	128 hPa (96 mm Hg)
· Density at 20°C (68 °F):	0.81587 g/cm ³ (6.808 lbs/gal)
· Relative density	Not determined.
· Vapour density	Not determined.
· Evaporation rate	Not determined.
· Solubility in / Miscibility with Water:	Soluble
· Segregation coefficient (n-octanol/water):	Not determined.
· Viscosity:	
dynamic:	Not determined.
kinematic:	Not determined.
· Solvent content:	
Organic solvents:	87.7 %
Water:	12.3 %
· Other information	No further relevant information available.

10 Stability and reactivity

- **Thermal decomposition / conditions to be avoided:** No decomposition if used according to specifications.
- **Incompatible materials:** Incompatible material: strong oxidizers.
- **Hazardous decomposition products:** No dangerous decomposition products known.

11 Toxicological information

- **Acute toxicity:**

· **LD/LC50 values that are relevant for classification:**

67-56-1 methanol

Oral	LD50	5628 mg/kg (rat)
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- **Primary irritant effect:**
- **on the skin:** No irritating effect.
- **on the eye:** No irritating effect.
- **Sensitization:** No sensitizing effects known.
- **Additional toxicological information:**
The product shows the following dangers according to internally approved calculation methods for preparations:
Toxic



12 Ecological information

- **Aquatic toxicity:** No further relevant information available.
- **Persistence and degradability** No further relevant information available.
- **Behavior in environmental systems:**
- **Bioaccumulative potential** No further relevant information available.
- **Ecotoxicological effects:**
- **Other information:**
The ecological effects have not been thoroughly investigated, but currently none have been identified.
- **Additional ecological information:**
- **General notes:** Water hazard class 1 (Self-assessment): slightly hazardous for water.

13 Disposal considerations

- **Waste treatment methods**
- **Recommendation**
Must not be disposed of with solid waste.
Dispose of material in accordance with federal (40 CFR 261), state and local requirements.
This product is not considered a RCRA hazardous waste.
- **Uncleaned packagings:**
- **Recommendation:** Disposal must be made according to state and federal regulations.
- **Recommended cleansing agent:** Water, if necessary with cleansing agents.

14 Transport information

· UN-Number	
· DOT, ADR, IMDG, IATA	UN1230
· UN proper shipping name	
· DOT, IMDG, IATA	METHANOL, solution
· ADR	1230 METHANOL, solution
· Transport hazard class(es)	
· DOT, IMDG, IATA	
	
· Class	3 Flammable liquids.
· Label	3+6.1
· ADR	
	
· Class	3 (FT1) Flammable liquids
· Label	3+6.1

· Packing group · DOT, ADR, IMDG, IATA	II
· Environmental hazards: · Marine pollutant:	No
· Special precautions for user · Danger code (Kemler):	Warning: Flammable liquids 336
· EMS Number:	F-E,S-D
· Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code	Not applicable.
· UN "Model Regulation":	UN1230, METHANOL, solution, 3 (6.1), II

15 Regulatory information

· SARA Section 355 (extremely hazardous substances)
None of the ingredients is listed.
· SARA Section 313 (specific toxic chemical listings)
67-56-1 methanol
· TSCA (Toxic Substances Control Act)
All ingredients are listed.
· California Proposition 65 - Chemicals known to cause cancer
None of the ingredients is listed.
· Chemicals known to cause reproductive toxicity for females:
None of the ingredients is listed.
· Chemicals known to cause reproductive toxicity for males:
None of the ingredients is listed.
· Chemicals known to cause developmental toxicity:
None of the ingredients is listed.
· Carcinogenic categories
· IARC (International Agency for Research on Cancer)
None of the ingredients is listed.
· NTP (National Toxicology Program)
None of the ingredients is listed.
· TLV (Threshold Limit Value established by ACGIH)
None of the ingredients is listed.

· **Product related hazard information:**

The product has been classified and marked in accordance with regulations on hazardous materials.

· **Hazard symbols:**

T Toxic

F Highly flammable

· **Hazard-determining components of labeling:**

methanol

· **Risk phrases:**

11 Highly flammable.

23/24/25 Toxic by inhalation, in contact with skin and if swallowed.

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· **Safety phrases:**

4 Keep away from living quarters.

- 7/9 *Keep container tightly closed and in a well-ventilated place.*
- 16 *Keep away from sources of ignition - No smoking.*
- 36/37/39 *Wear suitable protective clothing, gloves and eye/face protection.*
- 45 *In case of accident or if you feel unwell, seek medical advice immediately.*
- 60 *This material and its container must be disposed of as hazardous waste.*

· **National regulations**

· **Technical instructions (air):**

<i>Class</i>	<i>Share in %</i>
<i>Wasser</i>	<i>12.3</i>
<i>I</i>	<i>87.7</i>

- **Water hazard class:** *Water hazard class 1 (Self-assessment): slightly hazardous for water.*

16 Other information:

The above information is believed to be correct but does not purport to be complete and should be used only as a guide. The burden of safe use of this material rests entirely with the user.

12/06/2012