

FCM Wash buffer (1X): sc-3624

DESCRIPTION

The FCM Wash buffer (1X) can be used as a cell wash buffer for flow cytometry.

Product size: 125 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 4° C.

WARNING

FCM Wash buffer (1X) contains 0.1% sodium azide which is known to be toxic. Avoid contact with skin, eyes, and mucous membranes.

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.

8/27/2010

Intracellular FCM System: sc-3624



The Power to Question

MATERIAL SAFETY DATA SHEET

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name: Intracellular FCM System

Product Number: sc-45063

Supplier: Santa Cruz Biotechnology, Inc.
2145 Delaware Avenue
Santa Cruz, CA 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. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards

No known OSHA hazards

GHS Classification

Acute toxicity, Oral (Category 4)

Acute toxicity, Dermal (Category 5)

Acute aquatic toxicity (Category 3)

Chronic aquatic toxicity (Category 3)

GHS Label elements, including precautionary statements

Pictogram



Signal word

Warning

Hazard statement(s)

H302

Harmful if swallowed.

H313

May be harmful in contact with skin.

H412

Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P273

Avoid release to the environment.

HMIS Classification

Health hazard: 0

Chronic Health Hazard: *

Flammability: 0

Physical hazards: 0

NFPA Rating

Health hazard: 0

Fire: 0

Reactivity Hazard: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.

Skin May be harmful if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.

Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Sodium azidesolution

No ingredients are hazardous according to OHSA criteria.

4. FIRST AID MEASURES

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIREFIGHTING MEASURES

Conditions of flammability

Not flammable or combustible.

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for firefighters

Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products

Hazardous decomposition products formed under fire conditions - Nature of decomposition products not known.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.

Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and cleaning up

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.

Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place. Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection

Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Form	liquid	pH	no data available
Melting/freezing point	no data available	Boiling point	no data available
Flash point	no data available	Ignition temperature	no data available
Auto-ignition temperature	no data available	Lower explosion limit	no data available
Upper explosion limit	no data available	Vapor pressure	no data available
Density	1.00 g/mL at 20 °C	Water solubility	no data available
Relative vapor density	no data available	Odor	no data available
Odor Threshold	no data available	Evaporation rate	no data available
Partition coefficient n-octanol/water	no data available		

10. STABILITY AND REACTIVITY

Chemical stability

Stable under recommended storage conditions.

Possibility of hazardous reactions

no data available

Conditions to avoid

no data available

Materials to avoid

Heavy metals may form extremely explosive azides.

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50 no data available

Inhalation LC50 no data available

Dermal LD50 no data available

Other information on acute toxicity no data available

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

Eyes: no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)

no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)

no data available

Aspiration hazard

no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects

no data available

Additional Information

RTECS: Not available

12. ECOLOGICAL INFORMATION**Toxicity**

no data available

Bioaccumulative potential

no data available

PBT and vPvB assessment

no data available

Persistence and degradability

no data available

Mobility in soil

no data available

Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.
Harmful to aquatic life with long lasting effects.

13. DISPOSAL CONSIDERATIONS**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION**DOT (US)**

Not dangerous goods

IMDG

Not dangerous goods

IATA

Not dangerous goods

15. REGULATORY INFORMATION**OSHA Hazards**

No known OSHA hazards

SARA 302 Components

The following components are subject to reporting levels established by SARA Title III, Section 302:

Sodium azide

CAS-No.: 26628-22-8

SARA 313 Components

SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards

No SARA Hazards

Massachusetts Right To Know Components

Sodium azide

CAS-No.: 26628-22-8

Pennsylvania Right To Know Components

Water

CAS-No.: 7732-18-5

Sodium azide

CAS-No.: 26628-22-8

New Jersey Right To Know Components

Water

CAS-No.: 7732-18-5

California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

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.

6/13/2013