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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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SANTA CRUZ BIOTECHNOLOGY, INC.

FCM Fixation Buffer (10X): sc-3622

DESCRIPTION

The FCM Fixation buffer (10X) can be used to preserve hematopoietic cells prior to antibody-based intracellular protein labeling for flow cytometry.

Product size: 10 ml.

WARNING

FCM Fixation buffer (10X) contains formaldehyde (CAS# 30525-89-4). Formaldehyde is a suspected carcinogen and is toxic.

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 with FCM Fixation Buffer (sc-3622). Use 1 mL per million cells.

  NOTE: The 10x Fixation Buffer must be diluted to 1x with PBS prior to use.

- 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.

APPLICATION NOTES *cont.*

- 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 excercised 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.

  Decant supernatant and add approximately 50 ml 1X PBS to wash away any excess Permeabilization Buffer.

- Centrifuge for 5 minuntes 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.

APPLICATION NOTES *cont.*

- 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.
- 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.

RESEARCH USE

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

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