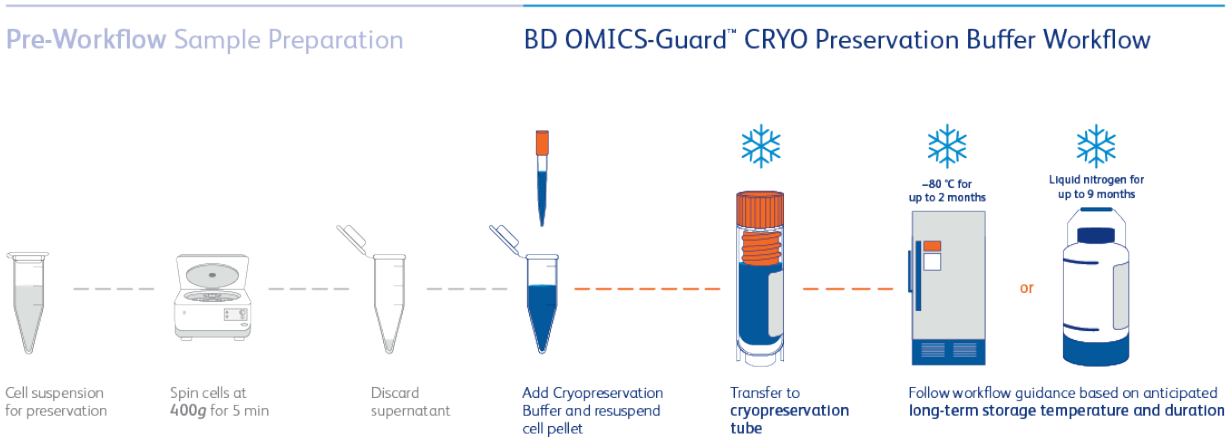
Long-term sample storage for single-cell suspensions with the BD OMICS-Guard™ CRYO Preservation Buffer

Workflow overview



Preparation

- Pre-cool (2–8 °C) the cell freezing container filled with isopropyl alcohol before use (e.g., Thermo Fisher Scientific Mr. Frosty™ Container, Cat. No. 5100-0001).
- Calculate the volume of BD OMICS-Guard™ CRYO Preservation Buffer needed to freeze ~1–2 million cells per mL of buffer. Note: use aseptic conditions while using the buffer by working in a biosafety cabinet.
- Storage duration and temperature: cells can be stored in BD OMICS-Guard™ CRYO Preservation Buffer at –80 °C for up to 2 months or in liquid nitrogen (below –130 °C) for up to 9 months.

Freezing cells in suspension

1. Collect single cells or dissociated cells in suspension and spin at 400*g* for 5 min.
2. Discard the supernatant.
3. Resuspend cells with the appropriate volume to achieve a ratio of ~1–2 x 10^6 cells per 1 mL of BD OMICS-Guard™ CRYO Preservation Buffer.
4. Transfer samples to cryopreservation tubes and put in a pre-cooled (2–8 °C) cell freezing container (e.g., Thermo Fisher Scientific Mr. Frosty™ Container). Prepare the unit per manufacturer's instructions.
5. Promptly put the cell freezing container in a –80 °C freezer overnight. Cells can be stored in a –80 °C freezer for up 2 months or transferred to liquid nitrogen (below –130 °C) for storage up to 9 months.

Thawing cells

1. Make the following thawing media for use during the thawing process. Use the same day it is made.
    i. Thawing media = complete RPMI media [RPMI1640 + 10% FBS (v/v)]

2. Pre-warm the thawing media to 37 °C and keep it at this temperature until use.
3. Remove frozen cells and immediately thaw in a 37 °C water bath for 1–2 min or just until the last small ice crystals remain.
4. To the 1-mL cell suspension, add 1 mL of warm (37 °C) thawing media for a total of 2-mL volume and gently pipette mix five times.
5. Transfer the 2-mL cell suspension to a 15-mL conical tube containing 8 mL of warm (37 °C) thawing media. Add cells slowly, then mix by gently inverting the tube.
6. Spin the tube at 400*g* for 5 min.
7. Aspirate the supernatant without disturbing the cell pellet.
8. Resuspend the cell pellet in 1-mL thawing media for an extra wash step (see steps 9–11 below) or resuspend cells in a buffer of choice for downstream applications.

Note: Steps 9–11 can be skipped if cell input is limited but may result in retention of cell debris.

9. Spin the tube at 400*g* for 5 min.
10. Aspirate the supernatant without disturbing the cell pellet.
11. Resuspend cells in the appropriate buffer needed for downstream applications.

Protocol for CITE-seq applications

BD OMICS-Guard™ CRYO Preservation Buffer allows antibody staining before or after sample preservation. For CITE-seq applications using BD® AbSeq Antibody-Oligos and/or BD® OMICS-One Protein Panels **after** sample preservation, use of the BD® AbSeq Enhancer Kit (Cat. no. 570750) is highly recommended. BD® AbSeq Enhancers can be added to the BD Fc Block™ Reagent step or used separately prior to single-cell staining with antibody-oligo cocktail.

Note: Staining with antibody-oligo panel **before** sample preservation or staining with the BD® Single-Cell Multiplexing Kits **only** after sample preservation does not require the use of the BD® AbSeq Enhancer Kit.

Staining protocol

1. Prepare the Human BD Fc Block™ Reagent as follows:

Component	Volume/sample (µL)	Volume/sample with overage (µL)
Stain buffer	65	78
BD Pharmingen™ Human BD Fc Block™	5	6
Total	70	84

2. To the 70 µL Human BD Fc Block™ Reagent mixture, add 10 µL of each of the three BD® AbSeq Enhancers for a total of 30 µL.
   Note: The Human BD Fc Block™ Reagent and BD® AbSeq Enhancers mixture should have a final volume of 100 µL.
3. Spin the cell suspension from the preserved sample at 800*g* and remove the supernatant without disturbing the pellet.
4. Add the 100 µL final mix and resuspend the cell pellet.
5. Incubate the cells at room temperature for 10 min.
6. For antibody-oligo staining:
   a. To stain with BD® OMICS-One Protein Panels, refer to the panel technical data sheet (TDS).
   b. To stain with BD® AbSeq Antibody-Oligo cocktails, refer to the standard AbSeq staining protocols (100 µL 2X BD® AbSeq Antibody-Oligo labeling MasterMix, incubate 30–60 min on ice). See BD

Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos Protocol (Doc ID: 23-24262). Note: for staining with >50-plex markers, the Fc Block™/BD® AbSeq Enhancer mix volume will need to be adjusted. Contact scomix@bd.com for more information.

Important modifications to the BD Rhapsody™ Single-Cell Cartridge workflow

For cells preserved in BD OMICS-Guard™ CRYO Preservation Buffer, ensure that the BD Rhapsody™ Cartridge workflow lysis step is at least 5 min long. Follow product protocol instructions if they indicate a lysis time longer than 5 min.

Ordering information

Description	Size	Cat. No.
BD OMICS-Guard™ CRYO Preservation Buffer	50 mL	628570
BD® AbSeq Enhancer Kit	Kit	570750

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