Cytodelics CRYO#20

CRYO#20

Summary:

CRYO#20 is a buffer specifically designed for long-term cryogenic preservation of fixed cells processed with Cytodelics *Whole blood processing kit* or *PBMCs fixation kit*. It might be used for cryogenic preservation of cells in suspension fixed with other products, however, testing might be required.

Recommended use:

1. CRYO#20 is recommended for cryogenic preservation of **fixed** leukocytes[1] or PBMCs/cultured cells[2].
2. The optimal volume per sample/aliquot is **250 – 500 µL** of CRYO#20 for freezing of 1.0-5.0 x 10^6 cells.
3. Staining with **fluorescently-labeled antibodies** is possible immediately after thawing in CRYO#20 buffer without a need for washing. However, it is recommended to test the conditions for each new antibody clone and/or new fluorescent tag.
4. CRYO#20 can be used for cryogenic preservation of **cells labeled** with fluorescently tagged antibodies.
5. Store CRYO#20 at room temperature.

DO NOT use for cryogenic preservation of LIVE CELLS!!!

DO NOT use as an alternative to Cytodelics Stabiliser!!!

---

[1] Isolated from whole blood with Cytodelics Whole blood processing kit. (catalog numbers: hC002-xxxx/mC002-xxxx/WBFL002-xxxx)

[2] PBMCs or other mammalian cell suspension containing no or limited number of RBCs fixed with Cytodelics PBMCs fixation kit (catalog numbers: PF002-xxx).

Cytodelics CRYO#20

Cytodelics

Protocol #1:
Freezing

1. After the last wash of the fixation protocol, aspirate the supernatant leaving **50-100µL** of the buffer on the bottom of the tube. Break the pellet and bring the fixed cells to suspension.
2. Add **250-500µL of the CRYO#20 buffer** to suspension of 1-5 x 10^6 cells, mix well.
3. Transfer to -80˚C freezer and store. Use of a freezing container is not needed.

Protocol #2:
Thawing

1. Set a **water bath** (or other alternative) to **30-37˚C** and wait until the temperature reaches the given interval.
2. Transfer cryogenically preserved samples to the water bath and quickly **thaw sample within 1-3 minutes.** Thawing for more than 5 minutes is not recommended.
3. (optional) Transfer sample quantitatively to centrifugation tube, add PBS (2/3 of the tube's nominal volume) and spin at **300g/5'-7'/@RT**. Aspirate the supernatant.
4. Sample is ready for further processing (e.g. staining with Ab-cocktail or acquisition).

Protocol #3:
Thawing & Staining without washing

1. Set a **water bath** (or other alternative) to **30-37˚C** and wait until the temperature reaches the given interval.
2. Transfer cryogenically preserved samples to the water bath and quickly **thaw sample within 1-3 minutes.** Thawing for more than 5 minutes is not recommended.
3. Spin at **300g/5'-7'/@RT** and aspirate the supernatant leaving 50-100µl of the buffer.
4. **Add an antibody cocktail** and stain **for 30-60 min** on ice.
5. **Wash** twice with 2-3 mL of cold PBS. Aspirate carefully to minimize cell loss.
6. Acquire the sample.