P09 – CRYO#20: freezing and thawing of fixed cells

CRYO#20 is a buffer for long-term cryogenic preservation of **fixed** cells processed with the Cytodelics Whole Blood Fix/Lyse Kit or the PBMC Fixation Kit. It may also be used for other fixed cell suspensions; in that case, testing is recommended.

Do NOT use CRYO#20 for cryogenic preservation of LIVE cells.
Do NOT use CRYO#20 as an alternative to the Cytodelics Stabiliser.

Recommended use

1. CRYO#20 is intended for **fixed leukocytes** (Whole Blood Fix/Lyse Kit: WBFL002, hC002, mC002) and for **fixed PBMCs or other cell suspensions** with no or few red blood cells (PBMC Fixation Kit: PF002).
2. Optimal volume per sample/aliquot: **250 – 500 µl of CRYO#20 for 1.0 – 5.0 × 10^6 cells**. For processed whole blood, 0.5 – 1-times the original blood volume (see the tables in P01 and P03).
3. Staining with fluorescently labelled antibodies is possible **directly after thawing** in CRYO#20, without washing. Test the conditions for each new antibody clone and/or fluorochrome.
4. Cells already labelled with fluorescently tagged antibodies can also be frozen in CRYO#20.
5. Store CRYO#20 at **room temperature**.

A. Freezing

1. After the last wash of the fixation protocol, aspirate the supernatant, leaving **50 – 100 µl** at the bottom of the tube. Break the pellet by vortexing and bring the fixed cells into suspension.
2. Add **250 – 500 µl of CRYO#20** to a suspension of 1 – 5 × 10^6 cells and mix well.
3. Transfer to a freezer at **≤ -80°C** and store. A freezing container is not needed.

B. Thawing

1. Set a water bath (or a heat block) to **30 – 37°C** and wait until it reaches the temperature.
2. Place the frozen samples in the water bath and **thaw quickly, within 1 – 3 minutes**. Thawing for more than 5 minutes is not recommended.

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3. *Optional wash:* transfer the sample quantitatively to a centrifugation tube, add PBS to approx. 2/3 of the nominal tube volume, centrifuge at **300g for 5 – 7 minutes** at room temperature and aspirate the supernatant.
4. The sample is ready for further processing (e.g., staining with the antibody cocktail or acquisition).

C. Thawing and staining without washing

1. Thaw the samples as described in B, steps 1 – 2.
2. Centrifuge at **300g for 5 – 7 minutes** at room temperature and aspirate the supernatant, leaving **50 – 100 µl**.
3. Add the antibody cocktail and stain:
    a. **overnight at 5 – 8℃** (fridge), tubes properly sealed to minimise evaporation, or
    b. **30 – 60 minutes on ice** (if time is a priority over quality).
4. Wash twice with **2 – 3 ml of cold PBS**. Aspirate carefully to minimise cell loss.
5. Acquire the sample.

Ordering information		
Product	**Product ID**	**Size**
CRYO#20	CR020-100	100 ml
Included in: Whole Blood Fix/Lyse Kit, combination kits	WBFL002, hC002, mC002	kit component
Included in: PBMC Fixation Kit / Gen2	PF002	kit component