P10 – Fixation of PBMCs and other cell suspensions

The PBMC Fixation Kit / Gen2 gently fixes purified cells (PBMCs, other primary cells, cell lines) and preserves their activation and phosphorylation state at an exact time point, for flow and mass cytometry. Fixed cells can be stained and acquired immediately, kept in the fridge for up to 3 days, or frozen in CRYO#20.

Preparation of kit components

1. **Fixation buffer:** mix **1 part of 2x Fix-Concentrate** with **1 part of Fix-Diluent** 5 – 10 minutes before use and let it reach room temperature. Unused fixation buffer can be stored for up to **24 hours at 4 – 8°C**.
2. **STOP and WASH buffers:** dilute the 4x STOP and 5x WASH concentrates with deionised water to 1x. The 1x buffers can be stored for **12 months at room temperature**.
3. **Storage of the kit:** Fix-Concentrate at **5 – 8°C**; Fix-Diluent, STOP, WASH and CRYO#20 at **room temperature**.

Fixation protocol

1. Transfer **2 – 5 × 10^6 PBMCs** to a 5 ml polystyrene "FACS" tube.
2. Centrifuge at **350 – 400g for 5 minutes**.
3. Aspirate the supernatant carefully. **Do not decant!**
4. Vortex to resuspend the pellet in the remaining volume (approx. 100 µl).
5. Add **0.5 ml of fixation buffer** and mix vigorously. Add the fixation buffer to each sample immediately after resuspension, so that cells do not wait in the residual volume.
6. Incubate at room temperature for **10 minutes**; gently vortex several times during the fixation if possible.
7. Add **0.6 ml of 1x STOP buffer**, vortex and incubate at room temperature for **10 – 15 minutes**.
8. Centrifuge at **400g for 5 minutes**.
9. Aspirate the supernatant, leaving some volume at the bottom. **Do not decant!**
10. Add **1 ml of 1x WASH buffer**.
11. Centrifuge at **400g for 5 minutes**.

12. Aspirate the supernatant, leaving some volume at the bottom. **Do not decant!**
13. Add **1 ml of 1x WASH buffer** and either:
    a. centrifuge at 400g for 5 minutes and proceed to step 14, or
    b. store the samples in the fridge (5 – 8˚C) for **up to 3 days**; then bring them to room temperature, centrifuge at 400g for 5 minutes and proceed to step 14.
14. Aspirate the supernatant, leaving some volume at the bottom. **Do not decant!**
15. Proceed with either:
    a. staining of the samples, or
    b. cryogenic preservation in **CRYO#20** at ≤ -80˚C for later processing (250 – 500 µl per 1 – 5 × 10^6 cells; see P09).

Recommendations

1. **Buffer-to-cell ratio:** keep the volume of the cell suspension to a minimum to avoid diluting the fixation buffer. Use 0.5 ml of fixation buffer for 2 – 5 × 10^6 PBMCs in approx. 100 µl, and 0.5 ml as the minimum volume even for fewer than 2 × 10^6 cells.
2. **Scaling up:** keep the ratios – 1 ml of fixation buffer for 4 – 10 × 10^6 PBMCs, and fixation buffer : STOP buffer = 1 : 1.2 (see the table).
3. **Fixation time:** 10 minutes is recommended; it can be varied from 5 to 20 minutes to optimise the fixation for a given application.
4. **Centrifugation:** with larger volumes, prolong centrifugation according to the liquid column height – from 5 minutes (full 5 ml tube or half-filled 15/50 ml tube) up to 10 minutes (full 15 or 50 ml tube). Do not increase the g-force.
5. **Tubes:** polystyrene tubes are preferred over polypropylene tubes to lower the risk of losing the cell pellet.

Recommended cell numbers and processing conditions					
PBMCs	**Fixation buffer**	**STOP buffer**	**WASH buffer**	**CRYO#20**	**Tube**
2 – 5 × 10^6	0.5 ml	0.6 ml	1.0 ml	250 – 500 µl	5 ml FACS tube (or 1.5 ml microtube)
4 – 10 × 10^6	1.0 ml	1.2 ml	2.0 ml	500 µl – 1.0 ml (or 2 aliquots)	5 ml FACS tube
8 – 40 × 10^6	2.0 – 4.0 ml	2.4 – 4.8 ml	4.0 – 6.0 ml	250 – 500 µl per aliquot of 1 – 5 × 10^6 cells	15 ml conical tube

Ordering information		
Product ID	**Kit**	**Enough for fixation of**
PF002-200	PBMC Fixation Kit / Gen2	2 × 10^9 cells
PF002-400	PBMC Fixation Kit / Gen2	4 × 10^9 cells
PF002-800	PBMC Fixation Kit / Gen2	8 × 10^9 cells
Kit components: 2x Fix-Concentrate, Fix-Diluent, 4x STOP buffer, 5x WASH buffer, CRYO#20.		

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