PBMCs fixation kit

Fixation protocol

1. Transfer $2*10^6$ – $5*10^6$ PBMCs to 5 mL FACS tube.
2. Centrifuge cells at **350-400g for 5min**.
3. Aspirate supernatant carefully. **Do not decant!**
4. Vortex samples to resuspend the pellet in the remaining volume.
5. Add **0.5 mL of fixation buffer** and mix vigorously. Do not wait until you add fixation buffer to the next sample.
6. Keep samples at room temperature for **10 minutes** and gently vortex multiple times during the fixation if possible.
7. Add **0.6 mL of STOP** buffer, vortex and incubate at room temperature for at **10-15 minutes**.
8. Centrifuge samples at **400g for 5 minutes**.
9. Aspirate supernatant but keep some volume at the bottom. **Do not decant!**
10. Add **1ml of WASH** buffer.
11. Centrifuge samples at **400g for 5 minutes**.
12. Aspirate supernatant but keep some volume at the bottom. **Do not decant!**
13. Add **1ml of WASH** buffer and either:
    a. centrifuge samples at **400g for 5 minutes** and proceed to step 14; or
    b. store samples in fridge for up to 3 days; then bring back to room temperature, centrifuge at 400g for 5 minutes and proceed to step 14.
14. Aspirate supernatant but keep some volume at the bottom. **Do not decant!**
15. At this point it is possible to either:
    a. proceed to staining of samples, or
    b. freeze samples at -80˚C or below in **Cryo#20** buffer for later processing.

Notes:

- Storage:
  - Keep the Fix-concentrate **at 5-8 ˚C.**
  - Keep Fix-dilution buffer, STOP, WASH and CRYO#20 buffers **at room temperature.**
- For processing of more cells, scale up all buffers' volumes to maintain ratios:
  - 1mL fix buffer for 4-$10*10^6$ PBMCs
  - [1:1.2] ratio for [fixation buffer volume: STOP buffer] volumes
- Polystyrene tubes are preferred over polypropylene ones to lower the risk of cell pellet loss.

Recommendations for preparation, storage and use of kit components

Fixation buffer

Prepare desired volume of fixation buffer by combining:

- 1 part of 2x Fix-concentrate
- 1 part of Fix-dilution buffer

5-10 min prior to use. Allow to reach room temperature.

Unused reconstituted fixation buffer can be stored for up to 24 hours at 4-8 ˚C.

Recommendations:

a) Fixation buffer-to-sample volume/cell number ratio
PBMCs fixation buffer ratio should be optimized to number of cells. However, volume of cells should be kept on minimum to avoid fix buffer dilution. We recommend to use 0.5 mL of fixation buffer for fixation of $2*10^6$ – $5*10^6$ PBMCs resuspended in approx. 100µL of PBS.
We recommend to use 0.5 mL of fixation buffer as the minimum volume even for cell counts lower than $2*10^6$.

b) Fixation time
Fixation for 10 minutes is recommended. However, fixation time can be varied from 5 to 20 minutes in order to optimize fixation process for the given application.

c) Centrifugation
When scaling up the number of processed cells, prolong centrifugation steps in regard to liquid column height in tube from 5 min (full 5mL FACS tube or half filled 15/50 mL tubes) up to 10 min (full 50 or 15 mL tubes). Do not increase RCF (relative centrifugation force; g-force)

STOP and WASH buffer

STOP and WASH buffers should be reconstituted with diH2O and can be stored for 12 months at room temperature.

Table 1: Recommended PBMCs numbers and corresponding processing conditions.

PBMCs	Fix buffer volume	STOP volume	WASH volume	CRYO#20 volume	Tube size
$2-5*10^6$	500 µL	600 µL	1.0 mL	50 µl	1.5 mL Eppendorf
$4-10*10^6$	1.0 mL	1.2 mL	2.0 mL	75-100 µl	5 mL FACS tube 5 mL Eppendorf
$8-40*10^6$	2.0 – 4.0 mL	2.4 – 4.8 mL	4.0 – 6.0 mL	100-500 µl	15 mL FALCON

Ordering information

Product cat# Component/volume	PF001-800 [ml]	PF001-400 [ml]	PF001-200 [ml]	
2x Fix-concentrate	400	200	100	
Fix-dilution buffer	400	200	100	
4x STOP buffer	250	125	65	
5x WASH buffer	700	350	180	
CRYO#20	100	50	25	
Suitable for up to	$8*10^9$	$4*10^9$	$2*10^9$	cells