

Staining of lymphocytes for flow cytometry

OVERVIEW

1. Thaw cells night before
2. Count and wash with PBS
3. Tetramer stain 10-30mins
4. Surface stain 30 mins in PBS with fixable blue (3 million cells → 200uL)
5. Fix/perm buff (diluted 1:3 in diluent) 30 mins
6. Intracellular stain in perm buff (diluted 1:10 in water) (3 million cells → 150uL) 60 mins

TRANSCRIPTION FACTOR PROTOCOL Total time: approx. 3 hrs. 35 mins

1. Rest cells overnight after thawing:
 - Suspend in R10 at 1 million cells per mL
 - Incubate overnight at 37 degrees 5% CO₂
2. Collect cells and re-count. Filter through 40um
3. Plate 3 million per well in 96 well plate
4. If staining with tetramer:
 - Spin 1600rpm for 3 mins and discard supernatant.
 - Wash 1X 200uL FACS buffer. Spin 1600rpm for 4 mins and discard supernatant.
 - Stain with tetramer (10uL per 3 million cells/ 60uL) for 10 mins RT
 - Wash with 200uL PBS and spin 1600rpm for 4 mins. Discard supernatant
5. Surface and viability staining. Make master mix, for one test of 3 million cells in 200uL
6. Incubate on ice for 30 mins
7. Spin 1600rpm for 4 mins and discard supernatant.
8. Wash with 200uL FACS buffer. Spin 1600rpm for 3 mins and discard supernatant
9. FIX/PERM, Make:
 - Fix/perm working solution: Dilute fix/perm concentrate (1 part) with fix/perm diluent (3 parts). 200uL working solution needed for each well.
 - Perm buffer: Dilute 10x concentrate with distilled water to make a 1x solution. 800uL working solution needed for each well.
 - Intracellular antibody master mix 1 test is 150 uL per 3 million cells
10. Add 200uL fix/perm working solution. Mix well.
11. Incubate in fix/perm in the dark 4C for 30-60 mins.
12. Spin 1600rpm for 4 mins and discard supernatant.
13. Wash with 200 uL Perm buffer. Spin 1800rpm for 4 mins and discard supernatant. Repeat.
14. Resuspend pellet in 150uL (3 million cells) perm buffer with intracellular antibodies and incubate in dark at RT for 60 mins
15. Wash with 200uL perm buffer. Spin 1800rpm for 4 mins and discard supernatant.
16. Wash with 200uL FACS buffer. Spin 1800rpm for 4 mins and discard supernatant.
17. Resuspend in 150-200uL FACS buffer and acquire on LSRII immediately.