Sample Preparation (PBMCs) Guidelines for Cytek® Mouse TBNK/Myeloid/Treg Kit

Contents

Introduction

For anyone working with **Cytek® TBNK/Myeloid/Treg Assay** to prepare and acquire mouse single cell suspension cells on Cytek Aurora cytometer, we recommend the following sample preparation instructions.

Import the 3 experiment templates: **TBNK Experiment Template, Myeloid Experiment Template and Treg Experiment Template** into SpectroFlo®.

** Please note that this kit is designed for research use only and is not for use in diagnostic or therapeutic procedures.*

** For best results, resuspend cells in stain buffer after staining and analyze samples on Aurora within 2 hours post staining.*

Materials

- Mouse cells in a single cell suspension
- Ghost Dye™ Violet 540, Tonbo Biosciences, 13-0879-T100
- Tonbo™ Mouse TBNK/Myeloid/Treg Kit
- PBS, pH7.4, Corning 21-040-CM
- Stain Buffer (BSA), BD Biosciences, 554657
- FoxP3 / Transcription Factor Staining Buffer Kit, (Tonbo, TNB-0607-KIT) (for Tube 3 (Treg tube) only)
- 12 x 75 mm tubes

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Sample Preparation

Preparing Ghost Dye™ Violet 540

Note: Ghost Dye™ Violet 540 is provided in solution prepared in anhydrous DMSO and should be protected from light and moisture. The dye is stable through 20 freeze/thaw cycles, if needed, aliquot smaller volumes and store at -20°C.

1. Prior to use, allow vial to equilibrate to room temperature.
2. Dilute the stock solution at 1:250 in PBS
3. Use the working solution at 5 µL per test (final dilution factor = 1:5000)

Protocol for Staining Cells in Tubes: TBNK and Myeloid Tubes (tubes 1 and 2)

Plan on using 300,000 cells for each Single Stain Reference Control and 0.5-2 million cells for each Multicolor Sample.

Please briefly centrifuge the reagent vials before use.

Viability Reference Control

1. Label a 12 x 75 mm tube for Viability Reference Control
2. Add ~300,000 cells to the tube
3. Add PBS to complete the final volume to 3 mL
4. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
5. Decant supernatant and blot on paper towel
6. Vortex thoroughly
7. Repeat steps (3)-(6) if the volume in step (2) is bigger than 1 mL
8. Add 5 µL of working solution Ghost Dye™ Violet 540 to the cell pellet
9. Vortex thoroughly
10. Incubate for 20 minutes at 2-8°C, protected from light
11. Add 3 mL of Stain Buffer
12. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
13. Decant supernatant and blot on paper towel
14. Vortex thoroughly
15. Resuspend in 300 µL Stain Buffer
    NOTE: Acquire at medium flow rate within 2 hours post staining

Single Stain Reference Controls

1. Label a 12 x 75 mm tubes for each Single Stain Reference Control
2. Add ~300,000 cells to each tube
3. Add Stain Buffer to complete the final volume to 3 mL
4. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
5. Decant supernatant and blot on paper towel
6. Vortex thoroughly
7. Repeat steps (3)-(6) if the volume in step (2) is bigger than 1 mL
8. Add 5 µL of appropriate monoclonal antibody to the cell pellet
9. Vortex thoroughly
10. Incubate for 30 minutes at 2-8°C, protected from light
11. Add 3 mL of Stain Buffer
12. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
13. Decant and blot on paper towel 14. Vortex thoroughly
15. Resuspend in 300 µL Stain Buffer

NOTE: Acquire at medium flow rate within 2 hours post staining (keeping cold and protected from light if not acquired right away)

Multicolor Sample

1. Label a 12 x 75 mm tube for each Multicolor sample
2. Prepare antibody cocktail in a 1.5 mL tube by adding 5 µL of the antibody reagents one by one.
   NOTE: *Prepare one extra test for the multicolor cocktail to take in account for any reagent loss in the process (ex. make multicolor cocktail for 6 tests if you have 5 multicolor samples to stain). Take 55 µL of the cocktail per multicolor sample and discard any leftover. Make antibody cocktails fresh each time before use.* ***DO NOT*** *re-use pre-made cocktails and* ***DO NOT*** *mix reagents from different kits*
3. Add ~1-2 million cells to Multicolor Sample tube
4. Add PBS to complete the final volume to 3 mL
5. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
6. Decant supernatant and blot on paper towel
7. Vortex thoroughly
8. Repeat steps (4)-(7) if the volume in step (3) is bigger than 1 mL
9. Add 5 µL of working solution Ghost Dye™ Violet 540 to the cell pellet
10. Vortex thoroughly
11. Incubate for 20 minutes at 2-8°C, protected from light
12. Add 3 mL of Stain Buffer
13. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
14. Decant and blot on paper towel
15. Vortex thoroughly
16. Add the antibody cocktail prepared in step (2)
17. Vortex thoroughly
18. Incubate for 30 minutes at 2-8°C, protected from light
19. Add 3 mL of Stain Buffer
20. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
21. Decant supernatant and blot on paper towel
22. Vortex thoroughly
23. Resuspend in 400 µL Stain Buffer
    NOTE: Acquire at medium flow rate within 2 hours post staining (keeping cold and protected from light if not acquired right away)

Protocol for Staining Cells in Tubes: Treg Tube (tube 3)

Plan on using 300,000 cells for each Single Stain Reference Control and 0.5-2 million cells for each Multicolor Sample.

Please briefly centrifuge the reagent vials before use.

Prepare working solutions of the follow reagents:

- Transcription Factor Fix/Perm Concentrate is supplied as a 4X stock solution and must be diluted with Transcription Factor Fix/Perm Diluent (1X) (TNB-1022-L160) prior to use. To prepare a 1X working solution, mix 1 part Transcription Factor Fix/Perm Concentrate (4X) with 3 parts Transcription Factor Fix/Perm Diluent (1X).
- Flow Cytometry Perm Buffer is supplied as a 10X stock solution and must be diluted to a 1X solution with distilled water prior to use.

Viability Reference Control

1. Label a 12 x 75 mm tube for Viability Reference Control
2. Add ~300,000 cells to the tube
3. Add PBS to complete the final volume to 3 mL
4. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
5. Decant supernatant and blot on paper towel
6. Vortex thoroughly
7. Repeat steps (3)-(6) if the volume in step (2) is bigger than 1 mL
8. Add 5 µL of working solution Ghost Dye™ Violet 540 to the cell pellet
9. Vortex thoroughly
10. Incubate for 20 minutes at 2-8°C, protected from light
11. Add 3 mL of Stain Buffer
12. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
13. Decant supernatant and blot on paper towel
14. Re-suspend pellets in 100 ul 1X Fix/Perm working solution
15. Incubate for 30 minutes at 2-8°C protected from light
16. Add 2 mL 1X Perm buffer, centrifuge at 500 x *g*, 5 minutes at 2-8°C
17. Decant supernatant and blot on paper towel
18. Vortex thoroughly
19. Repeat steps (16)-(18) one more time
20. Decant supernatant, leaving 100 uL of 1X Perm buffer, vortex thoroughly
21. Incubate for 30 minutes at 2-8°C, protected from light
22. Repeat steps (16)-(18) one more time, decant and blot on paper towel
23. Re-suspend stained cells in 300 uL Stain Buffer

NOTE: Acquire at medium flow rate within 2 hours post staining (keeping cold and protected from light if not acquired right away)

Single Stain Reference Controls

1. Label a 12 x 75 mm tubes for each Single Stain Reference Control
2. Add ~300,000 cells to each tube
3. Add Stain Buffer to complete the final volume to 3 mL
4. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
5. Decant supernatant and blot on paper towel
6. Vortex thoroughly
7. Repeat steps (3)-(6) if the volume in step (2) is bigger than 1 mL
8. Add 5 µL of appropriate monoclonal antibody for the surface markers to the cell pellet (do not add antibodies to the CD152 and FoxP3 tubes at this point, these will be added post permeabilization)
9. Vortex thoroughly
10. Incubate for 30 minutes at 2-8°C, protected from light
11. Add 3 mL of Stain Buffer
12. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
13. Decant supernatant and blot on paper towel
14. Re-suspend pellets in 100 ul 1X Fix/Perm working solution
15. Incubate for 30 minutes at 2-8°C protected from light
16. Add 2 mL 1X Perm buffer, centrifuge at 500 x *g*, 5 minutes at 2-8°C
17. Decant supernatant and blot on paper towel
18. Vortex thoroughly
19. Repeat steps (16)-(18) one more time
20. Decant supernatant, leaving 100 uL of 1X Perm buffer
21. Add the intracellular reagents 5 ul each (FoxP3 and CD152) to the appropriate reference control tubes

22. Incubate for 40 minutes at 2-8°C, protected from light
23. Repeat steps (16)-(18) one more time, blot on paper towel
24. Re-suspend stained cells in 300 uL Stain Buffer

NOTE: Acquire at medium flow rate within 2 hours post staining (keeping cold and protected from light if not acquired right away)

Multicolor Sample

1. Label a 12 x 75 mm tube for each Multicolor sample
2. Prepare antibody cocktail in a 1.5 mL tube by adding 5 µL of the surface antibody reagents (CD152 and FoxP3 will be added post permeabilization so do not add these 2 reagents at this point) one by one.
   NOTE: *Prepare one extra test for the multicolor cocktail to take in account for any reagent loss in the process (ex. make multicolor cocktail for 6 tests if you have 5 multicolor samples to stain). Take 40 µL of the cocktail per multicolor sample and discard any leftover. Make antibody cocktails fresh each time before use.* ***DO NOT*** *re-use pre-made cocktails and* ***DO NOT*** *mix reagents from different kits*
3. Add ~1-2 million cells to Multicolor Sample tube
4. Add PBS to complete the final volume to 3 mL
5. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
6. Decant supernatant and blot on paper towel
7. Vortex thoroughly
8. Repeat steps (4)-(7) if the volume in step (3) is bigger than 1 mL
9. Add 5 µL of working solution Ghost Dye™ Violet 540 to the cell pellet
10. Vortex thoroughly
11. Incubate for 20 minutes at 2-8°C, protected from light
12. Add 3 mL of Stain Buffer
13. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
14. Decant and blot on paper towel
15. Vortex thoroughly
16. Add the antibody cocktail prepared in step (2)
17. Vortex thoroughly
18. Incubate for 30 minutes at 2-8°C, protected from light
19. Add 3 mL of Stain Buffer
20. Centrifuge at 400 x *g*, 5 minutes at 2-8°C
21. Decant supernatant and blot on paper towel
22. Vortex thoroughly
23. Re-suspend pellets in 100 ul 1X Fix/Perm working solution
24. Incubate for 30 minutes at 2-8°C protected from light
25. Add 2 mL 1X Perm buffer, centrifuge at 500 x *g*, 5 minutes at 2-8°C
26. Decant supernatant and blot on paper towel
27. Vortex thoroughly
28. Repeat steps (25)-(27) one more time
29. Decant supernatant, leaving 100 uL of 1X Perm buffer
30. Add the intracellular reagents 5 ul each (FoxP3 and CD152)
31. Incubate for 30 minutes at 2-8°C, protected from light
32. Repeat steps (25)-(27) one more time
33. Decant supernatant and blot on paper towel
34. Re-suspend stained cells in 400 uL Stain Buffer

NOTE: Acquire at medium flow rate within 2 hours post staining (keeping cold and protected from light if not acquired right away)