Sample Preparation (PBMCs) Guidelines for Cytek® cFluor® MDSC Kit

Contents

Introduction

For anyone working with the **Cytek® cFluor® MDSC Kit** to prepare and acquire peripheral blood mononuclear cells (PBMCs) in a Cytek® Aurora or Northern Lights™ cytometer, here are Cytek's recommended sample preparation procedures*. These are 3 additional items to make your workflow easier:

1. Import the **Cytek® cFluor® MDSC Kit Tags** to the fluorescent tag lists in your SpectroFlo® Library section. If you already have existing tags in your library, delete them or overwrite them with the tags in this list.
2. Import experiment template "**Cytek® cFluor® MDSC Kit Template_PBMCs"** into the SpectroFlo® software.
3. Refer to **Cytek® cFluor® MDSC Kit , Acquisition Protocol** for a step-by-step guide for sample acquisition and analysis in the SpectroFlo® software.

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

Materials

Cytek® cFluor® MDSC Kit, Cytek Biosciences, Cat. R7-40010

- 15 Single reference reagents

Required but not supplied

- Frozen PBMCs
- Ghost Dye™ Violet 540, Cat. 13-0879
- RPMI Thermo Cat. 11875-093
- FBS Thermo Cat. A31604-01
- Stain Buffer (BSA) (BD Cat No. 554657)
- PBS, pH7.4, Corning 21-040-CM, or equivalent

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- Fixation Buffer, P/N: TNB-8222
- Cytek® FSP™ CompBeads, Cat. B7-10011
- Flow cytometer (Cytek® Northern Lights™, Cytek® Aurora™)
- Pipettes and pipette tips of 20 µL, 100 µL and 1000 µL
- 12 x 75 mm tubes or 96 well V-bottom deep plates (Corning 3960 or equivalent) and 96 well U-bottom polypropylene plates (Corning 3365 or equivalent)
- Vortex mixer

Sample Preparation

Thawing PBMCs

1. Pre-warm 50 mL RPMI with10% FBS at 37°C for at least 30 minutes
2. Thaw PBMC vial quickly in 37°C water bath until the core is loose
3. Transfer the cells into a 50 mL conical tube
4. Add 1 mL of warm media to the empty cryovial. Set it aside
5. Drop by drop, slowly add 10 mL of warm media to the cells in the 50 mL conical tube while gently swirling the tube to mix
6. Pour the content of the cryovial from step (4) into the 50 mL conical tube
7. Add additional media to complete the final volume to 20 mL
8. Centrifuge at 300 x *g*, 8 minutes
9. Decant the supernatant and blot on paper towel
10. Gently resuspend the pellet in 2 ml of warm media by pipetting up and down using a serological pipet
11. Repeat steps (7)-(10)
12. Resuspend in proper volume of Stain Buffer such that there is ~250,000 cells per 100µL of Stain Buffer and count cells
13. Loosen the cap on the 50 mL conical tube, place the cells in the cell culture incubator until ready to use

Preparing Viability Ghost Dye™ Violet 540

1. Dilute one part of Ghost Dye™ V540 with 9 parts of Stain Buffer to prepare the working solution of Ghost Dye™ Violet 540
2. Vortex to mix thoroughly
   ***NOTE**: Do not re-use the viability dye dilution*
3. Use the working solution at 1 µL per test

Protocol for Staining PBMCs in Tubes

Plan on using ~250,000 cells in a volume of 100µL of Stain Buffer for each Single Stain Reference Control (13 fluorescence, 1 Viability, 1 Unstained Control) and Multicolor Samples. When using bead samples ensure that an unstained beads control is created as well.

Viability Reference Control

1. Label a 12 x 75 mm tube for Viability Reference Control
2. Add ~250,000 (100uL) cells to the tube.
3. Add 1 µL of working solution Ghost Dye™ Violet 540 to the cell pellet.
4. Vortex thoroughly
5. Incubate for 15 minutes at room temperature, protected from light.
6. Add 2 mL of Stain Buffer
7. Centrifuge at 400 x *g*, 5 minutes at room temperature

8. Decant the supernatant and blot on paper towel.
9. Vortex thoroughly
10. Resuspend in 300 µL working solution of Fixation Buffer.
    NOTE: *Dilute Fixation Buffer 1:4 in PBS to make working fixative solution.*
11. Acquire at medium flow rate within 4 hours post staining.
    NOTE: *Samples may be stored at 4°C for 24 hours prior to acquiring on a cytometer.*

Single Stain Reference Controls

1. Label a 12 x 75 mm tubes for each Single Stain Reference Control
2. Add ~250,000 (100uL) cells to each tube. Use 1 drop of Cytek® FSP™ CompBeads Compensation Beads for applicable single-color reference controls.
   NOTE: *See Table 1 on page 4 for reference control type recommendations for each marker.*
3. Add correct amount of appropriate monoclonal antibody to the appropriate tubes (5µL/test for every reagent)
4. ***NOTE:*** *The single color reference control for cFluor R685 is a dump channel and should be CD3 R685, CD19 R685, and CD56 R685 included together in one sample tube.*
5. Vortex thoroughly
6. Incubate for 20 minutes at room temperature, protected from light.
7. For single stain and unstained cells, add 2mL of Stain Buffer into the tubes, mix briefly by vortex, centrifuge samples at 400 x *g* for 5 min, and then decant supernatant and blot on paper towel.
8. For single stain and unstained beads, add 2mL of Stain Buffer into the tubes, mix briefly by vortex, centrifuge samples at 600 x *g* for 6 min, and then decant supernatant and blot on paper towel.
9. For single color reference controls on beads repeat step 8.
10. Vortex thoroughly
11. Resuspend cell samples in 300 µL working solution of Fixation Buffer.
    NOTE: *Dilute Fixation Buffer 1:4 in PBS to make working fixative solution.*
12. Resuspend bead samples in 300 µL 1X PBS.
12. Acquire at medium flow rate within 4 hours post staining.
    NOTE: *Samples may be stored at 4°C for 24 hours prior to acquiring on a cytometer.*

Table 1. Reference Control Type Recommendations

NOTE: *Recommendations are for use with Cytek® FSP™ CompBeads, B7-10011.*

Laser	Target	Fluorochrome	Recommended Control Type
Violet	CD16	cFluor® V450	Beads
	CD15	cFluor®V505	Beads
	Viability	Ghost Dye™ V540	Cells
Blue	CD14	cFluor® B515	Cells or Beads
	CD45	cFluor® B548	Cells or Beads
	CD84	cFluor® BYG575	Cells or Beads
	CD11b	cFluor® BYG610	Cells or Beads
	CCR3(CD193)	cFluor® BYG667	Cells or Beads
	CD181	cFluor®BYG710	Beads
	CD33	cFluor® BYG781	Cells or Beads
Red	LOX-1	cFluor®R659	Beads
	CD3	cFluor® R685	Cells or Beads
	CD19		
	CD56		
	CD66b	cFluor® R720	Beads
	HLA-DR	cFluor® R840	Cells or Beads

Multicolor Sample

1. Label a 12 x 75 mm tube for each Multicolor sample.
2. Prepare antibody cocktail in a 12 x 75 mm tube. Add sufficient Ab (5µL/test for every reagent) for desired number of multicolor tests plus one extra.
   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). Make antibody cocktails fresh each time before use and* ***DO NOT*** *re-use pre-made cocktails.*
   NOTE: *For setting proper gates for LOX-1, CD181, and CD84, fluorescence-minus-one (FMO) multicolor stainings are recommended for these markers. To generate FMO samples, omit the addition of LOX-1, CD181, and CD84 from each FMO cocktails.*
3. Add ~250,000 cells to Multicolor Sample tube.
4. Add 1 µL of working solution Ghost Dye™ Violet 540 viability dye to the tube.
5. Vortex thoroughly
6. Incubate for 15 minutes at room temperature, protected from light.
7. Add 2 mL of Stain Buffer
8. Centrifuge at 400 x *g*, 5 minutes at room temperature
9. Decant supernatant and blot on paper towel.
10. Vortex thoroughly
11. Add appropriate amount of the antibody cocktail prepared in step (2)
12. Vortex thoroughly
13. Incubate for 20 minutes at room temperature, protected from light.
14. Add 2 mL of Stain Buffer
15. Centrifuge at 400 x *g*, 5 minutes at room temperature
16. Decant supernatant and blot on paper towel.
17. Vortex thoroughly

18. Resuspend in 300 µL working solution of Fixation Buffer.
    NOTE: *Dilute Fixation Buffer 1:4 in PBS to make working fixative solution.*
19. Acquire at medium flow rate within 4 hours post staining.
    NOTE: *Samples may be stored at 4°C for 24 hours prior to acquiring on a cytometer.*

For Research Use Only. Not intended for use in diagnostic procedures.

cFluor® B515, cFluor® B548, cFluor® R685, cFluor® R720, and cFluor® R840 are equivalent to CF®488A, CF®514, CF®660C, CF®700, and APC-CF®790T respectively, manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytek (LICENSEE). The manufacture, use, sale, offer for sale, or import of the product is covered by one or more of the patents or pending applications owned or licensed by Biotium. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.

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