Sample Preparation (Whole Blood) Guidelines for Cytek® cFluor® MDSC Kit

Contents

Introduction

For anyone working with the **Cytek® cFluor® MDSC Kit** to prepare and acquire whole blood cells 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®** Cytek® **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® MDSC Kit Template_Blood"** into the SpectroFlo® software.
3. Refer to the **Acquisition Protocol for Cytek® cFluor® MDSC Kit 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 color reagents

Required but not supplied

- Whole blood of no less than 2mL collected in tubes
- Cytek® cFluor® RBC Lyse/Fix Solution 10x, Cat. R7-60010
- Stain Buffer (BSA) (BD Biosciences, Cat No. 554657)
- Deionized Water
- PBS, pH7.4, Corning, Cat. 21-040-CM
- Fixation Buffer, Cat. 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
- Vortex mixer

Protocol for Staining Whole Blood in Tubes

Plan to use 100 µL whole blood for each Single Stain Reference Control (13 fluorescence and 1 Unstained Control), and 100 µL whole blood for each Multicolor Sample. Viability dye staining is usually not needed for fresh blood samples. When using bead samples ensure that an unstained beads control is created as well.

RBC Lyse/Fix Solution

Dilute 1 part of Cytek® RBC Lyse/Fix Solution 10X with 9 parts of room temperature deionized water to prepare 1X RBC Lyse/Fix Solution.
NOTE: *The 1X Lyse/Fix solution can be used up to 1 month from the date of preparation when stored at room temperature.*

Single Color Reference Controls

1. Label a 12 x 75 mm tube for each Single Stain Reference Control.
2. Add 100 µL of whole blood to each Single Stain Reference Control tube. Use 1 drop of Cytek® FSP™ CompBeads Compensation Beads for applicable single-color reference controls. ***NOTE:*** *See Table 1 on page 3 for reference control type recommendations for each marker.*
3. Add correct amount of appropriate monoclonal antibody to each tube (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 2 mL of 1x Lyse/Fix Solution into the whole blood reference control tubes, mix briefly by vortex, and incubate for at least 15 minutes at room temperature in the dark.
8. For single stain and unstained beads, add 2 mL of Stain Buffer into the tube.
9. Centrifuge all whole blood samples at 400 x *g* for 5 minutes at room temperature, and then decant supernatant and blot on paper towels.
10. Centrifuge all bead samples at 600 x *g* for 6 minutes at room temperature, and then decant supernatant and blot on paper towels.
11. Add 2 mL of Stain Buffer to each blood sample, Vortex briefly and centrifuge at 400 x *g* for 5 minutes at room temperature, and then decant supernatant and blot on paper towel.
12. Add 2 mL of Stain Buffer to each bead sample, vortex briefly and centrifuge at 600 x *g* for 6 minutes at room temperature, and then decant supernatant and blot on paper towel.
13. Resuspend blood samples in 300 µL working solution of Fixation Buffer.
    NOTE: *Dilute Fixation Buffer 1:4 in PBS to make working fixative solution.*
14. Resuspend bead samples in 300 µL 1X PBS.
15. 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.*

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 staining's are recommended for these markers. To generate FMO samples, omit the addition of LOX-1, CD181, and CD84 from each FMO cocktails.*

3. Add 100 µL of well-mixed EDTA anticoagulated whole blood to the bottom of Multicolor Sample tube.
4. Add appropriate amount of the cocktail mix to the bottom of the tube.
5. Vortex thoroughly.
6. Incubate for 20 minutes at room temperature, protected from light.
7. Add 2 mL of 1x Lyse/Fix Solution into the tube, mix briefly by vortex, and incubate for at least 15 minutes at room temperature in the dark.
8. Centrifuge all samples at 400 x *g* for 5 minutes and then decant supernatant and blot on paper towel.
9. Add 2 mL of Stain Buffer to each sample, vortex, and centrifuge at 400 x *g* for 5 minutes and then decant supernatant and blot on paper towel.
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.*

Table 1. Reference Control Type Recommendations for Single Color Reference Controls

Laser	Target	Fluorochrome	Recommended Control Type
Violet	CD16	cFluor® V450	Cells or Beads
	CD15	cFluor®V505	Cells or Beads
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	Cells or Beads
	CD33	cFluor® BYG781	Cells or Beads
Red	LOX-1	cFluor® R659	Beads
	CD3	cFluor® R685	Cells or Beads
	CD19		
	CD56		
	CD66b	cFluor® R720	Cells or Beads
	HLA-DR	cFluor® R840	Cells or Beads

NOTE: *Recommendations are for use with Cytek® FSP™ CompBeads.*

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.

Cytek® FSP™ CompBeads are developed and manufactured by Slingshot Biosciences, Inc., Emeryville, CA.