Sample Preparation (Bone Marrow Stain/Lyse/Fix) Guidelines for Cytek® 20-Color AML Panel

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Introduction

For anyone working with the **Cytek® 20-Color AML Panel** to prepare and acquire bone marrow cells in Cytek® Northern Light™ or Aurora cytometer equipped with violet, blue and red lasers or higher, here are Cytek's recommended sample preparation procedures*. These are 3 additional items to make your workflow easier:

1. Import the **Cytek® 20-Color AML Panel 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® 20-Color AML Panel Template"** into SpectroFlo®.
3. Refer to **Cytek® 20-Color AML Panel Acquisition Protocol** for a step-by-step guide for sample acquisition and analysis in SpectroFlo®.

** Please note that this kit is designed for research use only and is not for use in diagnostic or therapeutic procedures. Following method has only been tested in bone marrow collected in Heparin tube.*

** For best results, resuspend cells in stain buffer after staining and analyze samples on Northern Light™ with 3 Lasers or Aurora within 2 hours post staining. Fixation with 1% paraformaldehyde following the procedure described in this protocol on page 4 can be performed if acquisition needs to be done at a later time, however, be aware of possible changes in the MFI for some antigens as well as quantitative differences compared to fresh samples in the enumeration of some populations.*

Materials

- Fresh Bone Marrow collected in Heparin tubes
- Cytek® 20-Color AML Panel, cFluor® Reagent Kit (19C) (P/N R7-40009) and CD19 Monoclonal Antibody (SJ25C1), Super Bright™ 780, eBioscience™ (P/N 78-0198-42)
- Cytek® RBC Lyse/Fix Solution 10X, R7-60010
- PBS, pH7.4, Corning 21-040-CM
- Cell strainer, Corning, 40 µm, 07-201-430
- Stain Buffer (BSA), BD Biosciences, 554657
- Paraformaldehyde solution 4% in PBS, Tonbo™, TNB-8222
- Cytek® FSP™ CompBeads, B7-10011

Sample Preparation

Stain and lyse/fix Bone Marrow in Tubes

1. Collect bone marrow into Heparin tubes*
2. Filter through a 40-µm cell strainer, and count cells using the hematology analyzer or flow cytometer, adjust cell conc. around 10 x 10^6 cells/mL in Stain Buffer

NOTE: *If the cell count is > 20 x 10^6 cells/mL, dilute to* 10 x 10^6 cells/mL in Stain Buffer.

Plan on using ~400,000 cells for each Single Stain Reference Control (20 fluorescence, and 1 Unstained Control), and ~1 million cells for each Multicolor Sample

NOTE: For AML MRD evaluation, using ~ 10 x 10^6 cells for each Multicolor Sample (do not need to dilute the samples).

Single Color Reference Controls

1. Label a 12 x 75 mm tube for each Single Stain Reference Control
2. Add ~50 µL of filtered bone marrow or 1 drop of Cytek® FSP™ CompBeads to each Single Stain Reference Control tube
   NOTE: *See Table 1 on page 4 for reference control type recommendations for each marker*.
3. Add 5 µL of appropriate monoclonal antibody
4. Vortex thoroughly
5. Incubate for 20 minutes at room temperature, protected from light
6. For single stained cells add 2 mL 1X RBC Lyse/Fix solution
   NOTE: *Prepare 1X RBC Lyse/Fix Solution from 10X RBC Lyse/Fix solution with deionized water. For example, to make 50 mL add 5 mL of 10X RBC Lyse/Fix solution, and 45 mL of deionized water.*
7. Vortex the tube briefly to mix
8. Incubate in the dark for 15 minutes at room temperature
9. Centrifuge at 530 x *g*, 5 minutes at room temperature
10. Decant and blot on paper towel
11. Vortex thoroughly to resuspend the cell pellet
12. Add 3 mL of stain buffer to the tube
13. Centrifuge at 530 x *g*, 5 minutes at room temperature
14. Decant and blot on paper towel
15. Vortex thoroughly to resuspend the cell pellet
16. For single stain beads, wash twice by adding 2 mL of stain buffer (or PBS contain 1% BSA), centrifuging (at 600 x *g* for 6 minutes), and aspirating the supernatant leaving approximately 50 µL of supernatant in the tube each time.
17. Resuspend in 300 µL Stain Buffer or go to step (1) in "Cell Fixation in Tubes" on page 3 to fix the cells or beads in 1% paraformaldehyde

NOTE: *If the samples need to be stored at 4°C for more than 2 hours prior to collecting data, follow the steps in "Cell Fixation in Tubes" on page 4 to fix the samples in 1% paraformaldehyde*

18. Acquire at medium or high flow rate within 2 hours post staining if cells are not fixed

Multicolor Sample

1. Label a 12 x 75 mm tube for each Multicolor sample
2. Prepare antibody cocktail according to the number of Multicolor samples. Add 5 µL per test of each antibody.
   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 100 µL of the cocktail per multicolor sample and discard any leftover. Make antibody cocktails fresh each time before use and* ***DO NOT*** *re-use pre-made cocktails. Centrifuge the antibody cocktails at 8,000 -10,000 x g, 5 minutes at room temperature to avoid antibody aggregates. Take 100 µL supernatant per test.*
3. Add ~100 µL of filtered bone marrow to Multicolor Sample tube
4. Vortex thoroughly
5. Incubate for 20 minutes at room temperature, protected from light
6. Add 2 mL 1X RBC Lyse/Fix solution
7. Vortex the tube briefly to mix
8. Incubate in the dark for 15 minutes at room temperature
9. Centrifuge at 530 x *g*, 5 minutes at room temperature
10. Decant and blot on paper towel
11. Vortex thoroughly to resuspend the cell pellet
12. Add 3 mL of stain buffer to the tube
13. Centrifuge at 530 x *g*, 5 minutes at room temperature
14. Decant and blot on paper towel
15. Vortex thoroughly to resuspend the cell pellet
16. Resuspend in 300 µL Stain Buffer or go to step (1) in "Cell Fixation in Tubes" on page 3 to fix the cells in 1% paraformaldehyde
    NOTE: *If the samples need to be stored at 4°C for more than 2 hours prior to collecting data, follow the steps in "Cell Fixation in Tubes"* on page 4 *to fix the samples in 1% paraformaldehyde*
17. Acquire at medium or high flow rate within 2 hours post staining if cells are not fixed

Cell Fixation in Tubes

If the samples need to be stored at 4°C for more than 2 hours prior to collecting data, follow these steps to fix the samples in 1% paraformaldehyde and acquire within 24 hours post fixation.

1. Dilute 4% paraformaldehyde in PBS to make 1% paraformaldehyde solution
2. Add 300 µL of 1% paraformaldehyde to cell pellet.
3. Vortex thoroughly.
4. Incubate for 20 minutes at room temperature, protected from light
5. Add 3 mL of Stain Buffer
6. Centrifuge at 400 x g, 5 minutes at room temperature
7. Decant and blot on paper towel
8. Vortex thoroughly
9. Resuspend in 300 µL Stain Buffer for Single Stain Reference Controls and 400 µL for Multicolor Samples
10. Store at 4°C and acquire within 24 hours post fixation

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

Laser	Target	Fluorochrome	Recommended Control Type
Violet	CD16	cFluor® V420	Cells or Beads
	CD14	cFluor® V450	Cells or Beads
	HLA-DR	cFluor® V505	Cells or Beads
	CD4	cFluor® V547	Cells or Beads
	CD11b	cFluor® V610	Cells Only
	CD19	Super Bright™ 780	Cells or Beads
Blue	CD7	cFluor® B515	Cells or Beads
	CD15	cFluor® B548	Cells or Beads
	CD34	cFluor® BYG575	Cells Only
	CD33	cFluor® BYG610	Cells Only
	CD71	cFluor® BYG667	Cells Only
	CD38	cFluor® B690	Cells or Beads
	CD117	cFluor® BYG710	Cells or Beads
	CD56	cFluor® BYG750	Cells Only
	CD10	cFluor® BYG781	Cells or Beads
Red	CD13	cFluor® R659	Cells or Beads
	CD5	cFluor® R685	Cells Only
	CD123	cFluor® R720	Cells or Beads
	CD64	cFluor® R780	Cells or Beads
	CD45	cFluor® R840	Cells Only

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

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

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