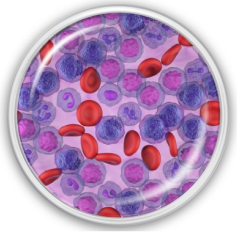

CYTEK DOC-00492 20-Color AML Panel User Guide

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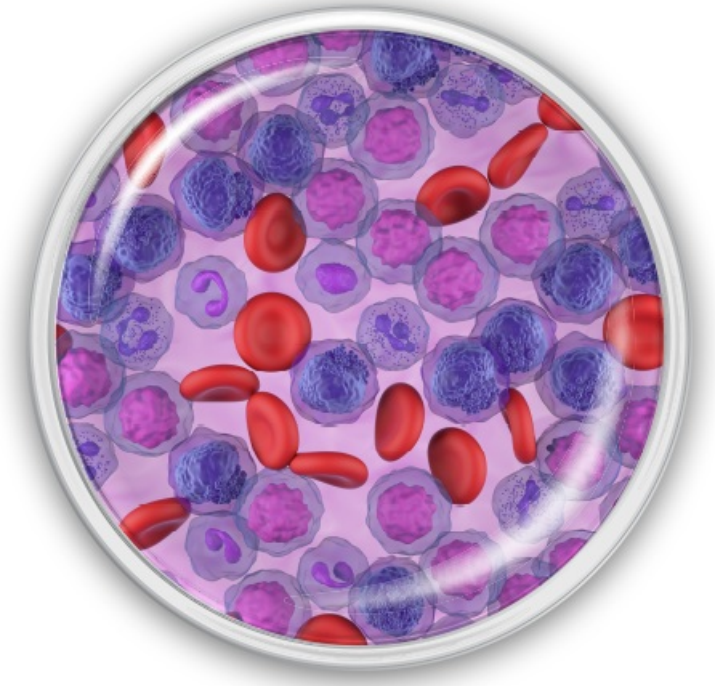

Sample Preparation (Bone Marrow Bulk Lyse) Guidelines for Cytek® 20-Color AML Panel

Contents

Introduction

For anyone working with the **Cytek® 20-Color AML** Panel to prepare and acquire bone marrow cells in the Cytek® Northern Lights™ 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-ViaDye™ Red**" into your SpectroFlo® library module.
3. Refer to **Cytek® 20-Color AML Panel** Acquisition Protocol for a step-by-step guide for sample acquisition and analysis in SpectroFlo® software.

* 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 a 3-laser Cytek Northern Lights™ system or Cytek Aurora system 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)
- ViaDye™ Red Fixable Viability Dye, Cytek Biosciences, R7-60008
- RBC Lysis Buffer, Tonbo™, TNB-4300
- Cell strainer, Corning, 40 µm, 07-201-430
- PBS, pH7.4, Corning 21-040-CM
- Stain Buffer (BSA), BD Biosciences, 554657
- Paraformaldehyde solution 4% in PBS, Tonbo™, TNB-8222
- Cytek® FSP ™ CompBeads, B7-10011

Sample Preparation

Bulk-lysing Bone Marrow

1. Collect bone marrow into Heparin tubes*
2. Prepare a fresh working 1X RBC Lysis Buffer containing 0.1% Paraformaldehyde. For example, to make 50 mL add 5 mL of 10X RBC Lysis Buffer, 1.25 mL of 4% Paraformaldehyde, and 43.75 mL of deionized water.
3. Transfer 13 mL of room temperature 1X lysis solution (with 0.1% Paraformaldehyde) into a 15 mL conical tube
4. Transfer 1 mL of well mixed bone marrow to the tube containing 13 mL of 1X lysis solution
5. Close and tighten the cap, mix gently by inverting or placing the tube on a tube rocker for 5 minutes
6. Centrifuge at 300 x g, for 5 minutes
7. Gently aspirate the supernatant without disturbing the pellet
8. Vortex gently
9. Add 10 mL of room temperature 1X lysis solution (with 0.1% Paraformaldehyde) to the pellet, mix well
10. Repeat steps (5)-(8)
11. Add 10 mL Stain Buffer, mix well
12. Centrifuge at 300 x g, for 5 minutes
13. Gently aspirate the supernatant without disturbing the pellet
14. Vortex gently
15. Repeat steps (11)-(14)
16. Resuspend in proper volume of Stain Buffer, filter through 40-µm cell strainer, and count cells using the hematology analyzer or flow cytometer, adjust cell conc. to be around 10 x 10^6 /mL in Stain Buffer

* Please note that only bone marrow collected in Heparin tubes has been tested using this method.

Preparing ViaDye™ Red Fixable Viability Dye

1. Completely thaw DMSO
2. Add 100 µL DMSO to the lyophilized ViaDye™ Red Fixable Viability Dye stock (=1 mM stock solution)
3. Vortex to mix thoroughly
4. Aliquot and freeze at -20°C until use
5. Thaw an aliquot of the stock solution at room temperature, protected from light, before each use.
   NOTE: Do not re-freeze or re-use the viability dye
6. Dilute the stock solution at 1:100 in PBS (=10 µM working solution)
   Use the working solution at 5 µL per test

Protocol for Staining Bulk-lysed Bone Marrow in Tubes

Plan on using ~400,000 cells for each Single Stain Reference Control (20 fluorescence, 1 Viability and 1 Unstained Control), and ~1 million cells for each Multicolor Sample
NOTE: For AML MRD evaluation, using ~ 10 million cells for each Multicolor Sample.

Viability Reference Control

1. Label a 12 x 75 mm tube for Viability Reference Control
2. Add ~400,000 cells to the tube
3. Add PBS to complete the final volume to 3 mL
4. Centrifuge at 530 x g, 5 minutes at room temperature
5. Decant supernatant and blot on paper towel

6. Vortex thoroughly
7. Add 5 µL of working solution ViaDye™ Red Fixable Viability Dye to the cell pellet
8. Vortex thoroughly
9. Incubate for 25 minutes at room temperature, protected from light
10. Add 3 mL of Stain Buffer
11. Centrifuge at 530 x g, 5 minutes at room temperature
12. Decant supernatant and blot on paper towel
13. Vortex thoroughly
14. Resuspend in 300 µL Stain Buffer or go to step (1) in "Cell Fixation in Tubes" on page 4 to fix the cells in 1% paraformaldehyde

    NOTE: If the samples need to be stored at 4°C for more than 2 hour prior to collecting data, follow the steps in "Cell Fixation in Tubes" on page 5 to fix the samples in 1% paraformaldehyde
15. Acquire at medium or high flow rate within 2 hours post staining if cells are not fixed

Single Color Reference Controls

1. Label a 12 x 75 mm tube for each Single Stain Reference Control
2. Add ~50 µL of lysed cells 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 25 minutes at room temperature, protected from light
6. For single stained cells add 3 mL of Stain Buffer
7. Centrifuge at 530 x g, 5 minutes at room temperature
8. Decant and blot on paper towel
9. Vortex thoroughly
10. For single stain beads, wash twice by adding 2ml of stain buffer (or PBS contain 1% BSA), centrifuging (6 minutes at 600g), and aspirating the supernatant leaving approximately 50µl of supernatant in the tube each time.
11. Resuspend cells or beads in 300 µL Stain Buffer or go to step (1) in "Cell Fixation in Tubes" on page 4 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
12. 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 ~1 million cells to Multicolor Sample tube
4. Add PBS to complete the final volume to 3 mL
5. Centrifuge at 530 x g, 5 minutes at room temperature
6. Decant supernatant and blot on paper towel
7. Vortex thoroughly
8. Add 5 µL of working solution ViaDye™ Red Fixable Viability Dye to the cell pellet
9. Vortex thoroughly
10. Add the antibody cocktail prepared in step (2)
11. Vortex thoroughly
12. Incubate for 25 minutes at room temperature, protected from light
13. Add 3 mL of Stain Buffer
14. Centrifuge at 530 x g, 5 minutes at room temperature
15. Decant supernatant and blot on paper towel
16. Vortex thoroughly
17. Resuspend in 300 µL Stain Buffer or go to step (1) in "Cell Fixation in Tubes" on page 4 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
18. 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.
cFluor® V547, cFluor® B515, cFluor® B548, cFluor® BYG610, cFluor® R685, cFluor® R720 and cFluor® R840 are equivalent to CF®405L, CF®488A, CF®514, PE- CF®596R, 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.
Super Bright™ and eBioscience™ are trademarks of Thermo Fisher Scientific.
Cytek® FSP® CompBeads are developed and manufactured by Slingshot Biosciences, Inc.
cFluor® BYG610, cFluor® BYG667, cFluor® BYG710, cFluor® BYG750, and cFluor® BYG781 are tandem dyes made with R-PE. cFluor® B690 is a tandem dye made with PerCP. cFluor® R780 and cFluor® R840 are tandem dyes made with APC. Caution – Tandem dyes may show changes in their emission spectra with prolonged exposure to light or fixatives.

their respective owners.

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Documents / Resources

	CYTEK DOC-00492 20-Color AML Panel [pdf] User Guide DOC-00492 20-Color AML Panel, DOC-00492, 20-Color AML Panel, AML Panel, Panel