Sample Preparation (Whole Blood) Guidelines for Cytek® cFluor® Human B Cell Monitoring Kit

Contents

Introduction

For anyone working with the **Cytek® cFluor® Human B Cell Monitoring Kit** to prepare and acquire whole blood cells on Cytek® cytometers, here are our recommended sample preparation procedures*. These are 3 additional items to make your workflow easier:

1. Import the **Cytek® 13C B cell Assay 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® B Panel Assay Template_Blood** into SpectroFlo®.
3. Refer to the **Acquisition Protocol for Cytek® cFluor® Human B Cell Monitoring Kit** (DOC-00458) for a step-by-step guide for sample acquisition and analysis in SpectroFlo®.

** Following method has only been tested in blood collected in EDTA, heparin, and Cyto-Chex® tubes.*

** For best results, after staining and fixation with 1% paraformaldehyde, analyze samples on a Cytek® cytometer within 4 hours. If needed, samples may be stored at 4°C and analyzed within 24 hours post staining.*

DOC-00491; Rev A; Effective Date: 11/29/2022

Materials

- Whole blood collected in EDTA, heparin, or Cyto-Chex® tubes
- Cytek® cFluor® B Cell Monitoring Kit, Cytek Biosciences, R7-40008
- Cytek® FSP™ CompBeads, B7-10011
- PBS, pH7.4, Corning 21-040-CM
- RBC Lysis Buffer, Tonbo™, TNB-4300
- Stain Buffer (BSA), BD Biosciences, 554657
- Paraformaldehyde solution 4% in PBS, Tonbo™, TNB-8222

Sample Preparation

Bulk-lysing Whole Blood

1. Collect whole blood into EDTA, heparin, or Cyto-Chex tubes
2. Prepare a fresh working reagent of 1X RBC Lysis Buffer by diluting 1:10 with deionized water.
   NOTE: *Make sure to use room temperature solutions. Cold solutions tend to cause the cells to clump and stick to the sides of the tube, which makes them more difficult to centrifuge into a pellet*
3. Transfer 45 mL of room temperature 1X lysis solution into a 50 mL conical tube
4. Transfer 5 mL of well mixed whole blood to the tube containing 45 mL of 1X lysis solution
5. Close and tighten the cap, mix gently by inverting or by placing the tube on a tube rocker for 5 minutes
6. Centrifuge at 400 x *g*, for 5 minutes
7. Gently aspirate the supernatant without disturbing the pellet
8. Vortex gently
9. Add 50 mL of room temperature 1X lysis solution to the pellet, mix well
10. Repeat steps (5)-(7)
11. For heparin tubes* proceed to step 14. For EDTA and Cyto-Chex tubes, vortex to resuspend the pellet and add 50 mL 1X PBS, mix well
12. Centrifuge at 400 x *g*, for 5 minutes
13. Gently aspirate the supernatant without disturbing the pellet
14. Resuspend in 1 mL of Stain Buffer
    ** Blood collected in heparin has significant loss of cells when washing after lysis due to difficulty in spinning the cells down into a pellet. It is recommended to proceed to re-resuspend in 1 mL stain buffer for staining following lysis.*

Staining Bulk-lysed Whole Blood in Tubes

Plan on using ~100 µL whole blood* for each Single Stain Reference Control (13 fluorescence stains and 1 unstained control), and ~400 µL whole blood* for each Multicolor Sample*. Viability dye staining is not recommended for fresh blood samples.

** The recommendations are for a volume of original whole blood prior to bulk lysis.*

Single Color Reference Controls

NOTE: *Either beads or cells can be used for Single-color Reference Controls. For Unstained only cells can be used.*

1. Label a 12 x 75 mm test tube for each Single Stain Reference Control
2. For unstained tube add 50 µL of lysed cells
3. Add 50 µL of lysed cells or one drop of Cytek® FSP™ CompBeads to each Single-color Stain Reference Control tube
   NOTE: *See Table 1 for reference control type recommendations for each marker.*
4. Add 5 µL of appropriate monoclonal antibody

5. Vortex thoroughly
6. Incubate for 20 minutes at room temperature, protected from light
7. Vortex and add 3 mL of Stain Buffer
8. Centrifuge at 400 x *g*, 5 minutes at room temperature
9. Decant or aspirate the supernatant
10. Repeat steps (7) to (9) for Cytek® FSP™ CompBeads, then resuspend beads in 200 µL Stain Buffer
11. Vortex and resuspend cells in 200 µL 1% paraformaldehyde
    NOTE: *Dilute 4% paraformaldehyde in PBS to make 1% paraformaldehyde solution*
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*

Multicolor Sample

1. Label a 12 x 75 mm test tube for each Multicolor sample
2. Prepare antibody cocktail according to the number of Multicolor samples. Add 5 µL per test of each antibody.
3. Add 200 µL of RBC lysed cells to Multicolor Sample tubes
4. Add 65 µL of the antibody cocktail prepared in step (2)
5. Vortex thoroughly
6. Incubate for 20 minutes at room temperature, protected from light
7. Vortex and add 3 mL of Stain Buffer
8. Centrifuge at 400 x *g*, 5 minutes at room temperature
9. Decant or aspirate the supernatant
10. Vortex thoroughly
11. Resuspend in 400 µL 1% paraformaldehyde
12. Acquire at medium or high 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
Blue	IgM	cFluor® B515	Cells or Beads
	CD4	cFluor® B532	Cells or Beads
	CD15	cFluor® B548	Cells or Beads
	CD38	cFluor® BYG575	Cells or Beads
	CD27	cFluor® BYG610	Cells or Beads
	CD14	cFluor® BYG667	Cells or Beads
	IgD	cFluor® BYG710	Cells or Beads
	CD19	cFluor® BYG781	Cells or Beads
Red	CD3	cFluor® R659	Cells or Beads
	IgG	cFluor® R668	Beads*
	CD8	cFluor® R685	Cells or Beads
	CD20	cFluor® R720	Cells or Beads
	CD45	cFluor® R780	Cells or Beads

*If cells must be used for the single-color control of cFluor R668 anti-IgG, since the IgG+ B cell population is small, it is recommended to use either cFluor R668 conjugated anti-human CD3 or CD4, or other antibodies for highly expressed markers.

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

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

cFluor® B515, cFluor® B532, cFluor® B548, cFluor® R668 cFluor® R685 and cFluor® R720 are equivalent to CF®488A, CF®503, CF®514, CF®647, CF®660C and CF®700 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.