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

Contents

Introduction

For anyone working with **Cytek® cFluor® Human B Cell Monitoring Kit** to prepare and acquire peripheral blood mononuclear cells (PBMCs) 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 **Cytek® B Panel Assay Template_PBMC** 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.*

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Materials

- Frozen PBMC
- ViaDye™ Red Fixable Viability Dye, Cytek Biosciences, R7-60008
- Cytek® cFluor® B Cell Monitoring Kit, Cytek Biosciences, R7-40008
- Cytek® FSP™ CompBeads, B7-10011
- Stain Buffer (BSA), BD Biosciences, 554657
- Paraformaldehyde solution 4% in PBS, Tonbo™, TNB-8222
- 12 x 75 mm tubes

Sample Preparation

Thawing PBMCs

1. Pre-warm ~50 mL RPMI (10% FBS) at 37°C for at least 30 minutes
2. Thaw PBMC vial quickly in 37°C water bath until the core is loose and transfer the cells into a 50 mL conical tube
3. Add 1 mL of warm media to the empty cryovial. Set it aside.
4. 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
5. Pour the contents of the cryovial from step (3) into the 50 mL conical tube
6. Add additional media for a final volume of 20 mL
7. Centrifuge at 300 x *g*, 8 minutes and decant or aspirate the supernatant
8. Gently resuspend the pellet in 2 mL of warm media by pipetting up and down
9. Add media for a final volume of 20 mL
10. Take out 200 µL to count the cells on a flow cytometer
11. Centrifuge at 300 x *g*, 8 minutes at room temperature and decant or aspirate the supernatant
12. Resuspend the pellet in the proper volume of room temperature Stain Buffer so that the concentration is 1 million cells per 100 µL, this will equal to using 50 µL/test for references and 100 µL/test for multicolor samples
    NOTE: *Plan on using ~500,000 cells for each Single Stain Reference Control (13 fluorescence stains, 1 viability stain and 1 unstained control), and ~1 million cells for each Multicolor Sample.*

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 1:500 in PBS (=2 µM working solution)
7. Use the working solution at 5 µL per test

Staining PBMCs and Beads in Tubes

Single Color Reference Controls

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

1. Label a 12 x 75 mm test tube for each unstained, ViaDye Red and Single-color Stain Reference Controls
2. For Unstained and ViaDye Red tubes add 50 µL of PBMCs
3. Add 50 µL of PBMCs 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 monoclonal antibody or ViaDye Red to the appropriate tubes
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 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*

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 100 µL of PBMCs to each Multicolor Sample tube
4. Add 65 µL of the antibody cocktail prepared in step (2) and add 5 µL of ViaDye Red to each tube
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 300 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

Laser	Target	Fluorochrome	Recommended Control Type
	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.