



Sample Preparation (Whole Blood) Guidelines for the Cytek® cFluor® Human Pan Leukocyte Kit, LNW

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Introduction

For anyone working with the **Cytek® cFluor® Human Pan Leukocyte Kit, LNW** to prepare and acquire whole blood cells in Cytek® Northern Lights™ or Aurora (3-laser V-B-R configuration or higher) cytometer, here are Cytek’s recommended sample preparation procedures. These are 3 additional items to make your workflow easier:

1. Import the **Cytek® cFluor® Human Pan Leukocyte Kit Tags** to the fluorescent tag lists in your SpectroFlo® Library section. If you already have existing tags in your library, click “Ok” to overwrite them with the tags in this list.
2. Import experiment template “**Cytek Human Pan Leukocyte Experiment Template**” into your SpectroFlo® library module.
3. Refer to **Acquisition Protocol for the Cytek® cFluor® Human Pan Leukocyte Kit** 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.*

Materials

Cytek® cFluor® Human Pan Leukocyte Kit, LNW, Cytek Biosciences, R7-40007

- 15 single color reagents (2 µL/test), 50 tests per vial

Required but not supplied

- Whole blood of no less than 0.5mL collected in K₂EDTA, Heparin, ACD or Cyto-Chex® BCT tubes
- Cytek® RBC Lyse/Fix Solution 10X, R7-60010
- Deionized water
- Cytek® FSP™ CompBeads, B7-10011
- Flow cytometer (Cytek® Northern Lights™ or Aurora)
- 1X PBS containing 1% BSA or Staining buffer containing BSA (BD Cat No. 554657) or FBS protein (BD Cat No. 554656)
- 20 µL, 100 µL and 1000 µL pipettes and appropriate pipette tips
- 12 x 75 mm tubes



- Vortex mixer

Protocol for Staining Whole Blood in Tubes

Refer to **Table 1** for detailed information on the use of cells or Cytek® FSP™ CompBeads for single color reference controls for each reagent. Plan on using 100 µL whole blood for multicolor samples, unstained control, and each single stain reference control. Cytek® FSP™ CompBeads should be used for cFluor® BYG575 anti-human CD34, cFluor® BYG610 anti-human CD123, cFluor® BYG667 anti-human CD193 (CCR3), and cFluor® BYG710 anti-human CD56. Cells are recommended for cFluor® B515 anti-human CD16.

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 and unstained control.
 2. Gently invert the blood tube 8-10 times to mix the blood sample.
 3. Add 100 µL of whole blood or 1 drop of Cytek® FSP™ CompBeads to each single stain reference and unstained control tube. (See **Table 1**)
 4. Add 2 µL of appropriate monoclonal antibody.
 5. Vortex briefly.
 6. Incubate for 20 min at room temperature, protected from light.
 7. For the single stain and unstained cells, add 0.9 mL of 1X RBC Lyse/Fix Solution into the tube, mix briefly by vortex, and incubate for 15 min at room temperature in the dark.
 8. For single stain and unstained beads, add 2 ml of 1X PBS containing 1% BSA into the tube. Centrifuge (6 min at 600 x g, and immediately aspirate the supernatant leaving approximately 50 µl of supernatant in the tube. Resuspend the bead pellet in 1X PBS at preferred volume.
- Note:** *Staining buffer containing BSA or FBS protein can also be used for washing.*
9. Preview the unstained cell control on your cytometer at low flow rate to optimize FSC and SSC gains.
 10. Acquire single stained cells at high flow rate within 6 hours post staining.

NOTE: *Samples may be stored at 4°C for up to 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 1.5 mL tube. For one multicolor sample, add 2 µL each of the 15 single color conjugates one by one and pulse vortex gently to mix.
- 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).*
3. By reverse pipetting, add 100 µL of well-mixed EDTA-anticoagulated whole blood to the bottom of multicolor sample tube. Avoid any blood touching the side of the tube.
 4. **NOTE:** *for accurate blood sample volume, reverse pipetting is recommended.*
 5. Add 30 µL of the cocktail mix to the bottom of the tube.
 6. Vortex briefly.
 7. Incubate for 20 min at room temperature, protected from light.
 8. Add 0.9 mL of 1X Lyse/Fix Solution into the tube, mix briefly by vortex, and incubate for 15 min at room temperature in the dark.

9. Before running multicolor samples, preview the *CD45-V547 Single Stain Reference Control* tube to set the proper CD45 (V8 channel) threshold to exclude CD45 negative populations and debris.
10. Then preview multicolor samples for 10 seconds at low flow rate to make sure the sample is running properly and then record the entire sample at high flow rate.
11. Acquire samples within 6 hours post staining. It will take approximately 15 min per tube.
NOTE: *Samples may be stored at 4°C for up to 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	CD8	cFluor® V450	Cells or Beads
	HLA-DR	cFluor® V505	Cells or Beads
	CD45	cFluor® V547	Cells or Beads
	CD4	cFluor® V610	Cells or Beads
Blue	CD16	cFluor® B515	Cells
	CD34	cFluor® BYG575	Beads
	CD123	cFluor® BYG610	Beads
	CD193 (CCR3)	cFluor® BYG667	Beads
	CD56	cFluor® BYG710	Beads
	CD19	cFluor® BYG750	Cells or Beads
	CD14	cFluor® BYG781	Cells or Beads
Red	CD7	cFluor® R659	Cells or Beads
	CD20	cFluor® R685	Cells or Beads
	CD66b	cFluor® R720	Cells or Beads
	CD3	cFluor® R780	Cells or Beads

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® R685 and cFluor® R720 are equivalent to CF® 405L, CF® 488A, 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.

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® R780 is a tandem dye made with APC. Caution – Tandem dyes may show changes in their emission spectra with prolonged exposure to light or fixatives.

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