

**Before you begin**: Start up the system and run the daily startup procedure. Collect the job aids listed above. You will use those to guide you through this workflow.

**Objective**: To isolate four subsets of lymphocytes with high purity.

**Sample description:** Peripheral blood mononucleated cells (PBMCs), ranging in size from about 5 to 14 µm, were prepared from whole blood by density centrifugation, stained with the chosen antibodies, and then fixed for biosafety. Single-color controls and an unstained control are included.

Fluorochrome	Label	Excitation/emission (nm)
V450	CD20	405/450
BV510	CD4	405/510
FITC	CD3	488/519
PerCP-Cy5.5	CD8	488/676
APC	CD56	638/660
APC-H7	CD45	638/782

# 1. Create experiment.

- a. Create a new blank experiment.
- b. Edit experiment name and description.

PERIMENTS > 6 CO	LOR SORT_AK		
Design Experiment	Select Imaging Features	Adjust Gains	Set Up Single-Stain Controls
EXPERIMENT INFO	RMATION		
Experiment Name:	6 color sort_AK	🟠 Use as	Experiment Template

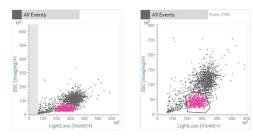
- c. Add appropriate fluorochromes and labels.
- d. Do not include an autofluorescence control.

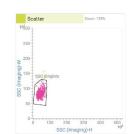
Autofluorescence	Control



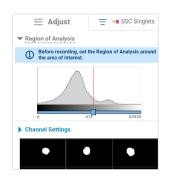
# 2. Adjust gains.

- a. Load the 6-color sample tube.
- b. Adjust the plot zoom, scatter gains, threshold, and gates to encompass lymphocytes.



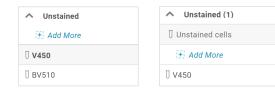


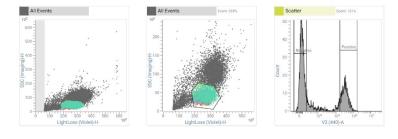
- c. Adjust the Region of Analysis properly for SSC Singlets.
- d. View the spectral plot and if any detectors are saturated, lower the gains.
- e. Unload the tube.



### 3. Set up single-stained controls.

- a. Add an Unstained control and name it.
- b. Ensure that the Region of Analysis is set correctly for the controls.
- c. Record data for each control tube.
- d. Adjust plot scaling and gate positions as needed and click **OK** to confirm each control.



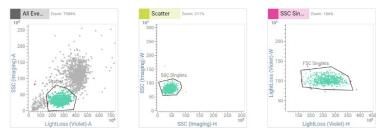


e. Verify that the Raw Mode indicator disappears when all tubes have been confirmed.

😮 BD	EXPERIMENTS > 6 COLOR SORT_AK		
Д	Design Experiment	$\geq$	Select Imaging

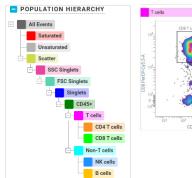
## 4. Record and analyze data.

- a. Load the sample tube.
- b. Adjust the plot zoom and scatter and singlet gates to encompass lymphocytes.



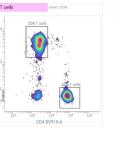
#### View data, continued

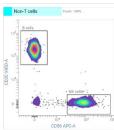
- c. Toggle off the Images Stored switch and record 10,000 events.
- d. Name the pre-sort data file.
- e. Create new plots to view populations of interest.
- f. Gate the appropriate populations. Rename the gates.



COLLECTION SETUP

4-Way Tub





# 5. Sort.

- a. In the Collection Setup panel, select 4-Way Tube,
  5.0 mL, and Purity.
- b. Assign populations to tubes.
- c. Assign 30,000 as the target event count for each tube.
- d. Install your collection device and close the sort chamber door.
- 5.0 mL Sort Mor Purity ٠ SORT SETUP Tube 1 2 3 0.50 mL ¢ Initial Buffer Volume 0.50 mL \$ 0.60 mL • 0.50 mL 4 Number of Events 30,000 30,000 30,000 \$ \$ Max: 703,125 event Max: 703.125 e Mar: 703,12 CD4 T cells CD8 T cells B cells

- e. Start the sort.
- f. Monitor the sort as it progresses. If needed, adjust the flow rate.
- g. When sorting finishes or is stopped, name the sort report.
- h. Perform a post-sort purity check on the View Data page.
  - i. Perform a backflush between tubes to minimize carryover.
  - ii. Toggle off the Images Stored switch and record 1,000 events.
  - iii. Record and name a post-sort data file for each collection tube.
  - iv. Use the Statistics panel to verify post-sort purity.



Population	Events 🙁	% Parent 😣	% Total 😣
CD4 T cells	968	99.79 %	96.80 %

#### Export and delete data. 6.

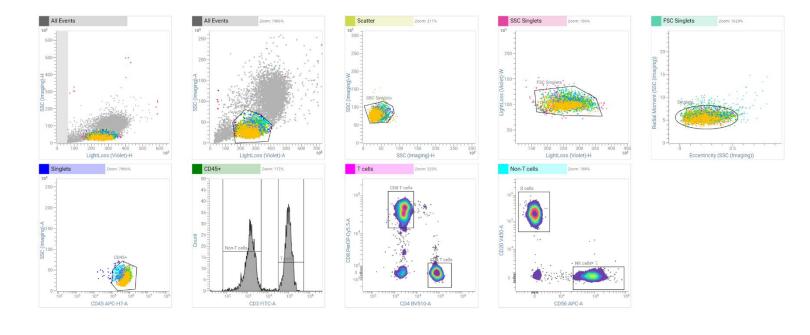
a. Export the report as a PDF on the View Reports page.

Select Sort Report: Tube sort • Export Report

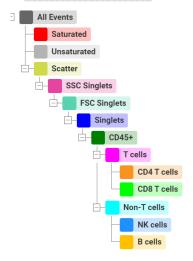
b. Export data files from the experiment.

PERIMENTS		Ex	port Data (12	2)			
New Experiment	Export Data (12)						
Display all user exp	eriments		All	Experiment	Created By	Events	FCS
ay an aver exp			NK post sort	6 color sort_AK	BD Bio	1,000	
	Name		B post sort	6 color sort_AK	BD Bio	1,000	
☆ 42	6 color sort_AK		CD8 post sort	6 color sort_AK	BD Bio	1,000	
			CD4 post sort	6 color sort_AK	BD Bio	1,000	
			6 color pre-sort	6 color sort_AK	BD Bio	10,000	
			APC-H7_001	6 color sort_AK	BD Bio CTRL	10,000	
			APC_001	6 color sort_AK	BD Bio CTRL	10,000	
			PerCP-Cy5.5_001	6 color sort_AK	BD Bio CTRL	10,000	
			FITC_001	6 color sort_AK	BD Bio CTRL	10,000	
			BV510_001	6 color sort_AK	BD Bio CTRL	10,000	
			V450_001	6 color sort_AK	BD Bio CTRL	10,000	
		~	Unstained cells_001	6 color sort_AK	BD Bio CTRL	10,000	

#### 6-color spectral sort example data



#### POPULATION HIERARCHY



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