Day 2 targeted workflow

6-color spectral sort

Start up system	→	Set up experiment	→	Establish settings	→	Sort and analyze	→	Manage data	→	Shut down system

- Create experiment

- Adjust settings
- Set up single-stain controls
- Record and analyze data

- Sort into tubes

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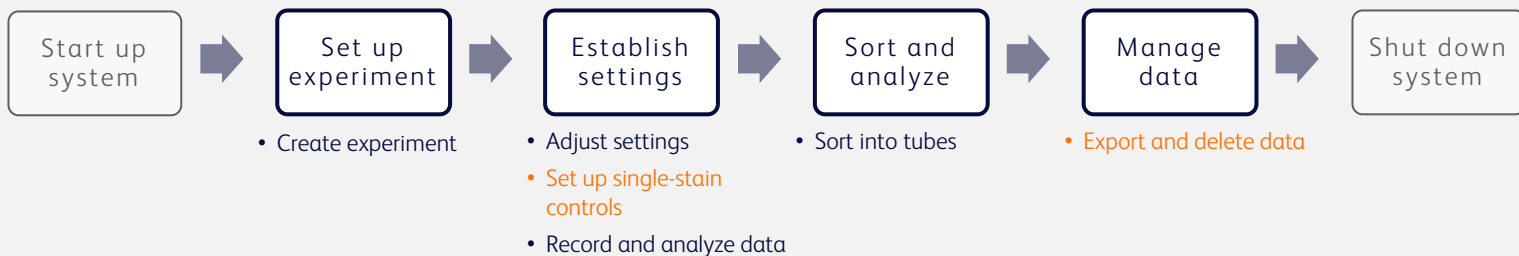
Before you begin: Start up the system and run the daily startup procedure. Collect the job aids listed above. You will use those to guide you through this workflow.

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.

    c. Add appropriate fluorochromes and labels.

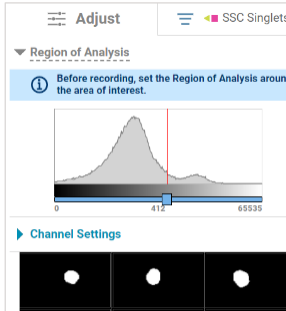   d. Do not include an autofluorescence control.

1

2. Adjust gains.

a. Load the 6-color sample tube.

b. Adjust the plot zoom, scatter gains, threshold, and gates to encompass lymphocytes.
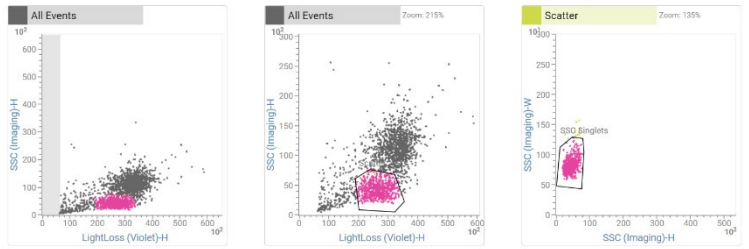

c. Adjust the Region of Analysis properly for SSC Singlets.

d. View the spectral plot and if any detectors are saturated, lower the gains.
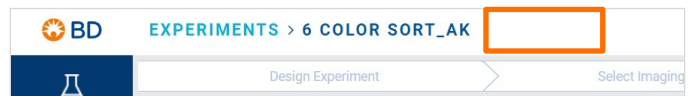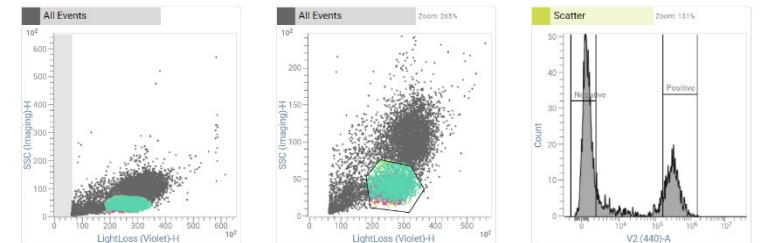
e. Unload the tube.

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.
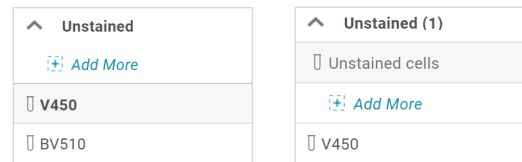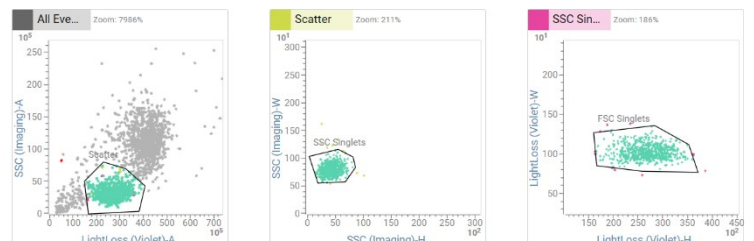

e. Verify that the Raw Mode indicator disappears when all tubes have been confirmed.
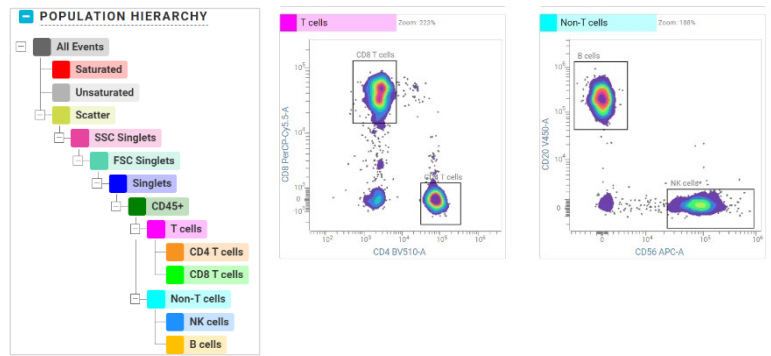

4. Record and analyze data.

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.

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.

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.

6. Export and delete data.

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

b. Export data files from the experiment.

6-color spectral sort example data
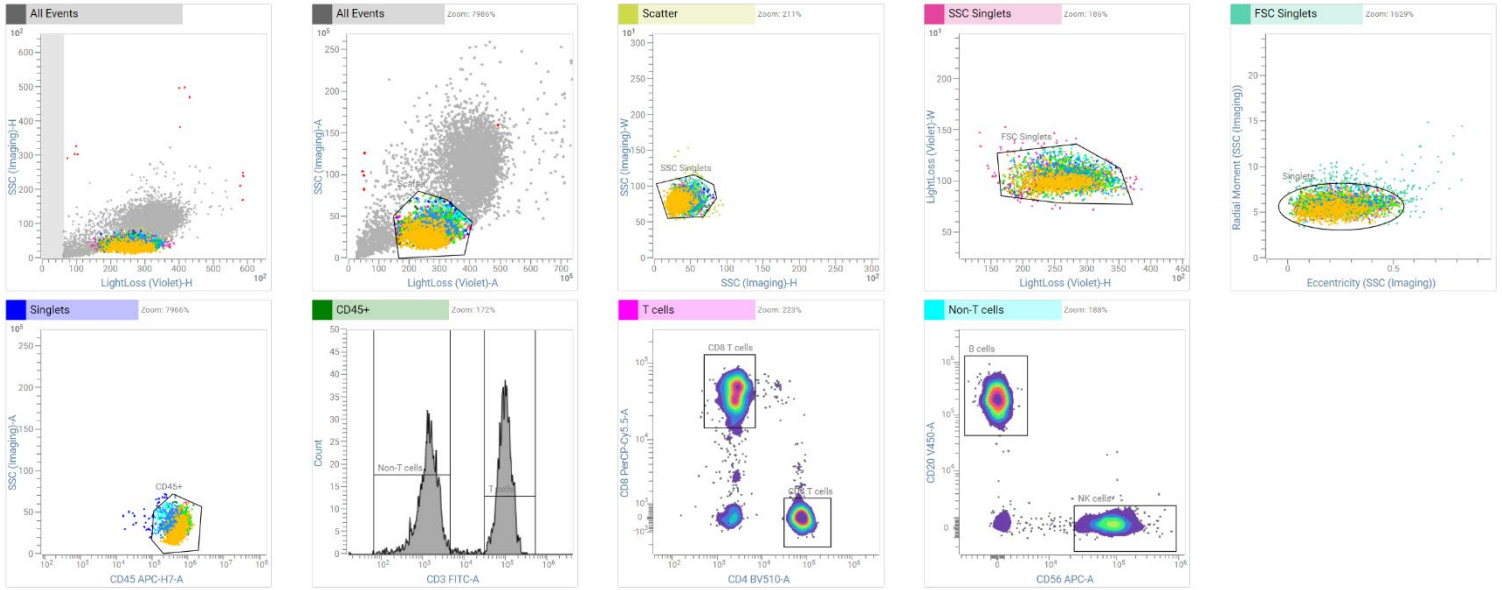
POPULATION HIERARCHY
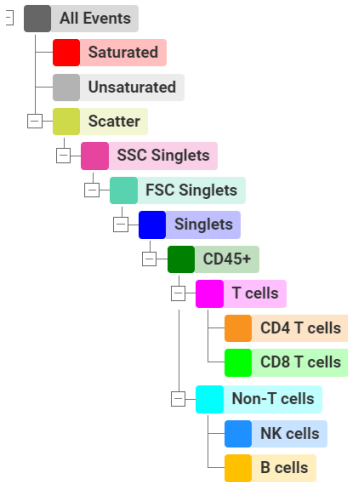
This material is for training purposes.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com