# Day 1 targeted workflow

## GFP basic sort

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

- Set up experiment: Create experiment
- Establish settings: Adjust settings; Record and analyze data
- Sort and analyze: Sort into tubes

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

**Objective**: To perform an enrichment sort on GFP-expressing cells.

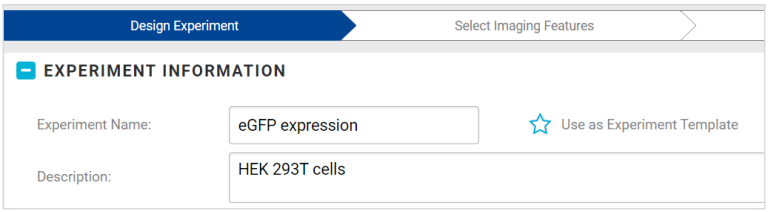
**Sample description:** HEK 293T cell line (human embryonic kidney), ranging from 11 to 15 µm, were transfected to express eGFP and fixed for biosafety.

| Fluorochrome | Excitation/emission (nm) |
|---|---|
| eGFP | 488/510 |

1. **Create experiment.**

   a. Create a new blank experiment.

   b. Enter experiment name and description.



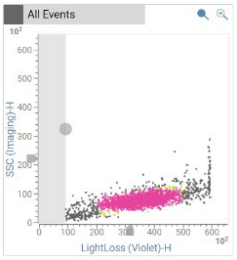

   c. Select eGFP.

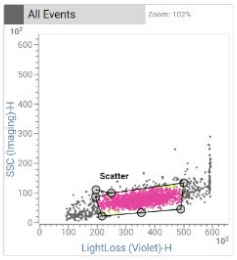

   d. Do not include an autofluorescence control.

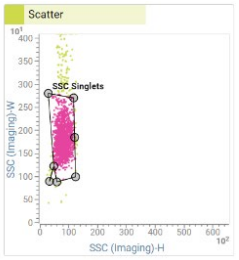
## 2. Adjust settings

a. Load the sample tube.

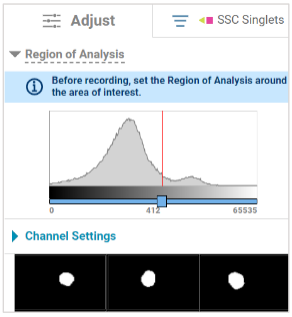
b. Adjust the plot zoom, scatter gains, threshold, and gates to encompass the cells.




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



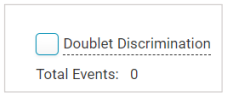

d. View the spectral plot and if any detectors are saturated, lower the gains.

e. Unload the tube.

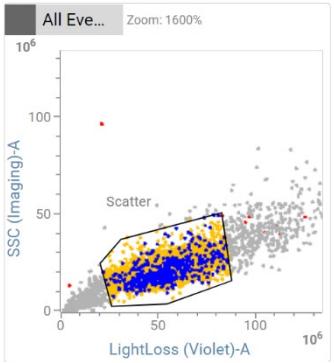

## 3. Record and analyze data.

a. Load the sample tube.

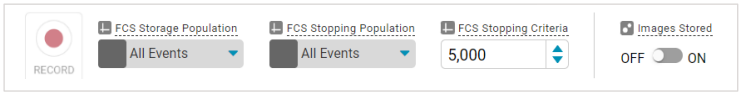
b. Clear the doublet discrimination checkbox.




c. Adjust the plot zoom and the scatter gate to encompass the cells.



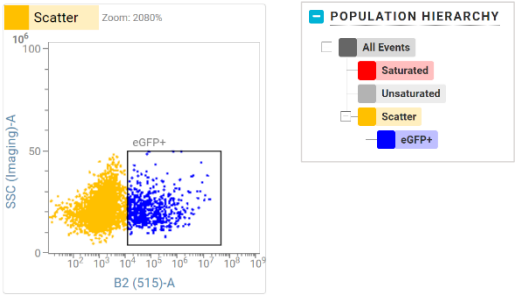
d. Toggle off the Images Stored switch and record 5,000 events.




e. Name the data file.

f. Create a new plot to view the population of interest.

g. Gate the eGFP positive population. Rename the gate eGFP+.




## 4. Sort.

a. In the Collection Setup panel, select **2-Way Tube, 5.0 mL**, and **Yield.**

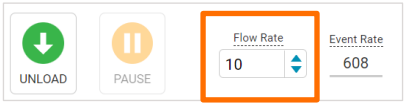
b. Assign the eGFP+ population to tube 1.

c. Assign 30,000 as the target event count for tube 1.

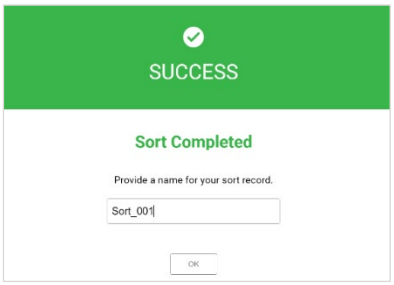
d. Place a tube in position 3 in the collection device, install the device, and close the sort chamber door.

e. Start the sort.


f. Monitor the sort as it progresses. If needed, adjust the flow rate.



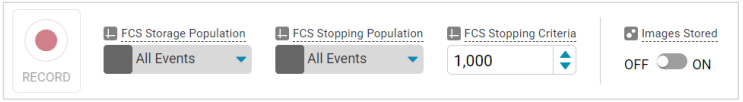

g. When sorting finishes or is stopped, name the sort report.




h. (Optional) Perform a post-sort purity check on the View Data page.

   i. Perform a backflush between tubes to minimize carryover.

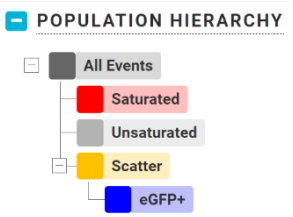
   ii. Toggle on or off the Images Stored switch and record 1,000 events.

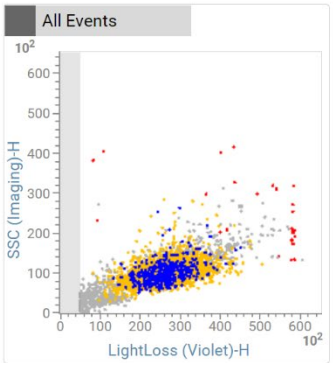


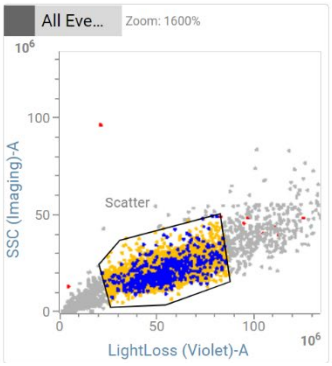

   iii. Record and name a post-sort data file for each collection tube.

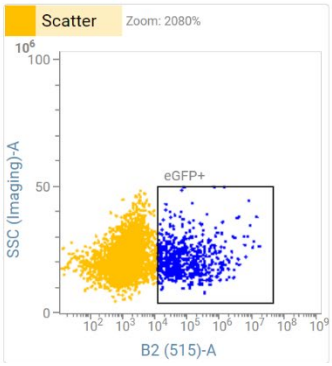
   iv. Use the Statistics panel to verify post-sort purity.

## GFP basic sort example data




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**