

Presort Information Sheet for Students

Name:

PI:

Date:

Type of Cell:

Note: Your original sample should be in a volume of at least 0.5ml and at a concentration of 5×10^6 to 5×10^7 per ml. Cell sorted onto plates can be at a lower concentration.

Faster sort speeds sacrifice recovery. Normal sorts result in >95% purity. If you can live with a less pure sort (sometimes in the case of rare events) be sure to tell us.

Please select sorting requirements:

Sorting into: _____ tubes _____ multiwell plate
Cells are _____ Robust _____ Fragile _____ Bacteria
Cell diameter _____ <15 μ m (blood cells and normal TC cells) _____ 15-25 μ m (large)
Cells need to be sterile _____ yes _____ no
Temperature _____ RT _____ 4°C _____ 37°C

List all fluorochromes:

Have you run this type of sample on the benchtop cytometers? _____ Yes _____ No

What approximate % of the original population do you want to separate in the sort?

_____ % Left _____ % Right

Calculate the following and then review this with your advisor.

1. How many sorted cells do you need at the end? ie. What is the stop number?

_____ Left _____ Right

2. Actual # of cells you need to start with _____

Post staining and post filtering cell counts are the most accurate.

Calculate the Irrational best number of starting cells to give the needed sorted cells:

$$IB_{\text{Starting}} = \text{Needed sorted cells} \div \%$$

Example: I need 100,000 sorted cells. My population is 2% of total cells.

$$\text{Starting} = 100,000 \div 0.02$$

$$\text{Starting} = 2,000,000$$

Multiply this by 2 to give a rough guess of the Actual number of cells you need to run through the sorter. You may need even more cells if they are fragile or sticky or if you are sorting rapidly. Filtering clumpy cells can result in considerable cell loss.

3. Calculate the length of time for the sort _____

$$\text{Time} = \text{Starting number} \div \text{Flow rate}$$

On the Influx a fast sort is 20,000/sec, medium sort is 10,000/sec, slow sort is 5000/sec.

This rate will be determined by your cell concentration and whether your cells are small and robust enough to be sorted under high-speed conditions.