

How to Judge the Length of a Sort

Representative Examples

Type of Sort	<i>Maximum</i> Sample Conc. (per ml)	Optimal Sort Rate (events / sec)	Throughput Per Hour	Approximate Efficiency
Single color >70% positive	20x10 ⁶ /ml	10,000-15,000	~30-45x10 ⁶	~80%
Single color, ~30-70% Positive	10x10 ⁶ /ml	~10,000	~30x10 ⁶	~70%
Single color, <20% Positive	10x10 ⁶ /ml	~5000-8000	~15-25x10 ⁶	~50-70%
2-Color, 2-Way	10x10 ⁶ /ml	~5000-8000	~15-25x10 ⁶	~70%
Multi-color, Multigate, 1-5% Pos	10x10 ⁶ /ml	~5000-8000	~15-25x10 ⁶	~50-70%

Tips:

1. The more complex the sort, or the lower the percent positive cells, the longer it will take to sort a given number of cells.
2. Samples that are more concentrated than the recommendations listed above will have lower yield and lower purity than properly diluted samples.
3. Running complex samples at a faster throughput greatly decreases yield, and can adversely affect purity.
4. Rule of thumb: # sorted cells needed ÷ % positive cells ÷ sort efficiency (from table above)= approximate # of cells to sort. Divide # of cells to sort by throughput per hour from above table to get a rough length of sort. Add an extra 10 minutes per sample if sorting multiple samples.

Example 1:

An investigator needs 5x10⁶ EGFP+ cells from each of 6 samples. The samples are ~50% positive. She would need to sort approximately 15x10⁶ cells per sample to ensure obtaining this number of cells:

$$5 \times 10^6 \div 0.5 \text{ (50\% positive)} \div 0.7 \text{ (70\% efficiency)} = 14.3 \times 10^6$$

At a throughput of ~ 30x10⁶ cells per hour, it will take approximately 30 minutes per specimen, plus 5-10 minutes between each specimen for handling, labeling, cleaning the sample aspirator, data storage, printing, and making a new data file. Therefore, 6 samples would take approximately 3.5-4 hours.

Example 2:

An investigator needs 2x10⁶ lin-/7AAD-/ckit+ stem cells, which represent ~2% of the total cells in his specimen. He would need to sort ~150-200x 10⁶ cells:

$$2 \times 10^6 \div .02 \text{ (2\% pos)} \div 0.5-0.7 \text{ (50-70\% eff.)} = 143-200 \times 10^6 \text{ cells}$$

At a throughput of ~25x10⁶ per hour, it would take 6-8 hours of sort time for this sample. If preparation takes several hours so that sorting cannot start until afternoon, then both sorters would need to be scheduled for the entire afternoon.