How to estimate the time needed to sort on a BD instrument

Different parameters will affect the time needed for your sort.

You will have to consider:

- which nozzle to use
- how fast the cells can be sorted
- how many samples need to be sort
- how many fluorochromes are in the panel
- what will be the collection vessels (plates take longer to align than regular tubes)
- does a post sort need to be done to prove the quality of the sort
- how many cells need to be recovered for the downstream purpose. If a specific number of cells are needed at the end of the sort, you will need to estimate the total number of cells needed to begin with:
  [(# of cells to recover) / (% of target cells in the sample)] **x 2** = # of total cells to sort
- time to calibrate and clean the instrument (an extra hour needs to be added for any sorting appointment)

Here is a list of what you should consider when you are evaluating how many cells to begin with and the speed for your sort.

1) **The tissue origin and/or the size of your cell.**
   The tissue origin will affect the nozzle used for the sort.
   In general cells coming from spleen, lymph node, blood and bone marrow can be sorted on high speed optimal rates. Cells from other tissue such as liver or intestine are in general run at lower speeds.

   Cells in suspension that are < 14 um are run using the 70 um nozzle and the 100 um nozzle is used for cells that are < 20 um when in suspension. We rarely sort on the 130 um nozzle in our core.

   Some cell treatments can increase the size and/or fragility of your cells. A larger nozzle may be needed after those types of treatments.

2) **The quality of your samples.**
   A sample that has a tendency to clump will take longer as the sample will need to be unloaded and filtered more often or we will have to remove a clog from the instrument (which can take 30 min to clear in some case). Filter the sample **right before sorting** to break up clumps.

3) **The concentration of the samples.**
   The concentration of your cells should be around 10 to 20 x 10^6 cells/ml **after staining**. If the total number of cells is < 5 x 10^6, resuspend in a minimum of 250 uL for your sort.

4) **Calculate time needed to sort using the tables with optimal values:**
   Note: The values in the tables are based on ideal samples that have gone through dead cell depletion and enrichment for target cells. Samples with a lot of debris or aggregates will take longer and have a lower efficiency. All sorts vary from one cell type to another.

   Please, contact us if you have any questions or for help figuring sorting time.

A. **Low Speed:**

Cells prepared from tissue such as intestine or liver will take longer due to their sticky nature and maybe run at a lower rate. Please remember, these are estimates and your sample may take more or less time.

		70 um Nozzle	85 um Nozzle	100 um Nozzle
	Cell size	< 14 um	< 17 um	< 20 um
	Suggested cell concentration	10 x 10^6 cells/mL	10 x 10^6 cells/mL	10 x 10^6 cells/mL
Every sort time calculation must include the shaded steps to get an estimate of time needed to sort.				
1.	**Instrument Setup/Calibrate**	30 min	30 min	30 min
2.	**Time to setup panel**			
	Time to set up < 8-color panel	30 min	30 min	30 min
	Time to set up > 8-color panel	1 hour	1 hour	1 hour
3.	**Time to set up tube/plate**			
	Time to set up tubes	5 min	5 min	5 min
	Time to set up 96-well plates	5 min	5 min	5 min
	Time to set up slides	5 min	5 min	5 min
	Time to set up Terasaki plate	10 min	10 min	10 min
4.	**Acquisition**			
	Maximum number of events during acquisition at maximum event rate	7,000 events/sec 0.42x10^6 events/min	5,000 events/sec 0.30x10^6 events/min	3,000 events/sec 0.18x10^6 events/min
	Maximum number of events sorted **in 1 hour**	25 x 10^6 events/hr	18 x 10^6 events/hr	10 x 10^6 events/hr
	Time to run **1 x 10^6 events**	2.5 min	3.5 min	5.5 min
	Maximum volume run in **1 hour** at max event rate	2.5 mL/hr	1.8 mL/hr	1.0 mL/hr
	Time needed to run **250 uL** of sample	6.0 min	8.5 min	14.0 min
5.	**Time to change samples & collection tubes**	2 min/samples	2 min/samples	2 min/samples
6.	**Time to post sort each collected tube for purity. (Optional)**	5 min	5 min	5 min
7.	**Cleaning the instrument**	30 min	30 min	30 min

Examples of calculated sorting times:

For 8 samples of 10 x 10^6 cell each (conc. 10 x 10^6 cell/mL) on a 70 um nozzle with a 7-color panel, collection into tubes and no post sort to check purity:
[30 min (instrument setup)] + [30 min (panel setup)] + [5 min (tube setup)] + [25 min x 8 (acquisition)] + [2 min x 8 (changing tubes)] + [30 min (clean instrument)] = **311 min = 5 hours and 11 minutes**

For 8 samples of 250 ul each on a 70 um nozzle with a 7-color panel:
[30 min (instrument setup)] + [30 min (panel setup)] + [5 min (tube setup)] + [6 min x 8 (acquisition)] + [2 min x 8 (changing tubes)] + [30 min (clean instrument)] = **159min = 2 hours and 39 minutes**

B. High Speed:

Cells isolated from the spleen, lymph node, blood, and bone marrow may be run at these optimal rates. Please remember, these are estimates and your sample may take more or less time.

		70 um Nozzle	85 um Nozzle	100 um Nozzle
	Cell size	< 14 um	< 17 um	< 20 um
	Suggested cell concentration	20 x 10^6 cells/mL	20 x 10^6 cells/mL	20 x 10^6 cells/mL
Every sort time calculation must include the shaded steps to get an estimate of time needed to sort.				
1.	**Instrument Setup/Calibrate**	30 min	30 min	30 min
2.	**Time to setup panel**			
	Time to set up < 8-color panel	30 min	30 min	30 min
	Time to set up > 8-color panel	1 hour	1 hour	1 hour
3.	**Time to set up tube/plate**			
	Time to set up tubes	5 min	5 min	5 min
	Time to set up 96-well plates	5 min	5 min	5 min
	Time to set up slides	5 min	5 min	5 min
	Time to set up Terasaki plate	10 min	10 min	10 min
4.	**Acquisition**			
	Maximum number of events during acquisition at maximum event rate	18,000 events/sec 1.08x10^6 events/min	11,000 events/sec 0.66x10^6 events/min	7,000 events/sec 0.42x10^6 events/min
	Maximum number of events sorted **in 1 hour**	64 x 10^6 events/hr	40 x 10^6 events/hr	25 x 10^6 events/hr
	Time to run **1 x 10^6 events**	1.0 min	2.0 min	2.5 min
	Maximum volume run in **1 hour** at max event rate	3.2 mL/hr	1.9 mL/hr	1.2 mL/hr
	Time needed to run **250 uL** of sample	5 min	8 min	12 min
5.	**Time to change samples & collection tubes**	2 min/sample	2 min/samples	2 min/samples
6.	**Time to post sort each collected tube for purity. (Optional)**	5 min	5 min	5 min
7.	**Cleaning the instrument**	30 min	30 min	30 min

Examples of calculated sorting times:

For 8 samples of 10 x 10^6 cell each (conc. 20 x 10^6 cell/mL) on a 70 um nozzle with a 7-color panel, collection into tubes and no post sort to check purity:

[30 min (instrument setup)] + [30 min (experiment/panel setup)] + [5 min (tube setup)] + [10 min x 8 (acquisition)] + [2 min x 8 (changing tubes)] + [30 min (clean instrument)] = **191 min = 3 hours and 11 minutes**

For 8 samples of 250 ul each on a 70 um nozzle with a 7-color panel:

[30 min (instrument setup)] + [30 min (experiment/panel setup)] + [5 min (tube setup)] + [5 min x 8 (acquisition)] + [2 min x 8 (changing tubes)] + [30 min (clean instrument)] = **151 min = 2 hours and 31 minutes**