flowkon
Flow Cytometry

LSRFortessa™ – HTS Loader Settings

Default HTS loader settings are provided for each throughput mode. You will need to optimize these settings for the plate type and assay you are running. You cannot change HTS settings during acquisition or when a sequence is in process.

HTS Settings

Setting	Standard Mode		High Throughput Mode	
	Default	Range	Default	Range
Sample flow rate [μl/sec]	1	0.5-3.0	1	0.5-3.0
Sample Volume [μl]	10	2-200	2	2-10
Mixing Volume [μl]	100	5-100	50	5-100
	180	25-250	200	25-250
Number of mixes [cycles]	2	0-5	2	0-5
Wash volume [μl]	400	200-800	200	200-800

Table I: HTS default settings. The HTS can process a 96-well plate in approximately 44 minutes in standard mode and approximately 15 minutes in high-throughput mode using the default settings listed in the table.

Sample Volume

The Sample Volume is the volume of sample aspirated from each well for acquisition. In standard mode, the HTS aspirates the selected sample volume plus an additional 20 μl from the well. In HTS mode the HTS aspirates a fixed volume of 22 μl per well, even though you can select a sample volume between 2-10 μl.

Note: Make sure each well contains sufficient sample for the entered volume. Insufficient volume can introduce air bubbles into the system.

Standard Mode

☑ The available maximum sample volume can be calculated as follow:
   Volume pipetted into well - Aspirated excess (20 µl) - Dead volume (20 µl)

Tipp: To make sure you do not run out of sample, BD recommends that you prepare your plate with a minimum of 250 µL of sample/well for a 96-well plate in standard mode, 100 µL/well for a 96-well plate in high throughput mode, and 50 µL/well for a 384-well plate (either mode).

Mixing Volume

Note: Make sure each well on your plate contains sufficient sample for mixing. A mixing volume that is larger than the available volume introduces air bubbles into the sample.

☑ Volume one-half the sample volume is recommended

Speed and Number of Mixes

☑ Use default values as a starting point

Note: Adjust the speed and number of mixes to obtain the most homogeneous population for acquisition, which is impacted by the viscosity of the sample in the well. Increasing the mixing speed and volume might improve throughput and mixing efficiency, but it could also introduce air bubbles in the sample well. In addition, increased mixing speed could compromise the separator bubble between the sample and sheath, resulting in sporadic event rates and possibly higher carryover.

Wash Volume

The Wash Volume is the volume of sheath fluid dispensed for rinsing between wells. You can vary the amount of wash volume to minimize carryover. In general, a greater wash volume results in less carryover, but the greater the volume, the slower the system throughput.

☑ Volume double of the sample volume and a minimum of 400-µL wash volume
   is recommended.