Salk Flow Core Reference Sheet: BD Influx

DESCRIPTION: The Salk core has 2 state-of-the-art BD Influx™ cell sorters. These are jet-in-air design instruments uniquely suited for isolating delicate cells, and for high-speed sorting. "Influx-1" is equipped with 5 lasers, 11 fluorescent detectors; "ShadowFACS" is equipped with 4 lasers, 13 fluorescent detectors (both units can detect forward- and side scatter light signals). Additionally, ShadowFACS is equipped with a forward scatter pinhole assembly that facilitates detection of dim FSC signals when performing small particle analysis. Both Influxes have biosafety enclosures that enable BSL2 rated sorts. Several features of the Influx (including flow cell characteristics and use of low-noise pre-amp electronics) come together to enable excellent detection sensitivity and stable high-throughput sorting, while maintaining the integrity and viability of sensitive cell types. It is also remarkably fast and accurate at sorting into 384-well plates (~3.5min/plate for highly abundant targets). *This is a hands-on instrument requiring manual alignment. Due to the level of commitment, training for autonomous use is only available for high usage Salk users: enquire at* ***fccf@salk.edu***

INFLUX-1 CONFIGURATION:

*** Scatter parameters are off the 488nm laser**

Laser Name	Filter	Examples of Fluorescent Labels that can be used
488nm 200mW	692/40 BP	PerCP-Cy5.5, BB700
	530/40 BP	FITC, AF488, GFP, YFP, Zombie-Green
640nm 120mW	750 LP	APC-Cy7, Zombie NIR
	670/30 **BP**	APC, AF647
561nm 150mW	750 LP	PE-Cy7
	670/30 BP	PE-Cy5
	593/40 BP	PE, tdTomato, AF594, RFP, TMRE, TMRM, PE-Dazzle
355nm 100mW	460/50 BP	Zombie-UV, Hoechst-Blue, DAPI
	670/30 BP	Hoechst-Red
405nm 100mW	520/35 BP	BV510, LD Aqua, CFP
	460/50 BP	DAPI, Pacific Blue, BV421, Hoechst, Zombie Violet, BFP, Dylight 405

OPERATING SOFTWARE: Sortware, version 1.2.0.142

NOZZLES: 70, 86, 100, 140-um

TEMPERATURE CONTROL: Both sample and collection

SORT COLLECTION: Up to 6 sorting streams can be generated (i.e. can sort up to "6-way") while a single sample is being run in the system. This requires the "cooperation" of the sample; it must be suitable quality with minimal stickiness to work at all, and sort with good purity. Up to 6-way sorting possible for 1.5 and 2ml eppendorf or 5ml FACS tubes. 15ml and 50ml tubes are limited to 2-way sorting. 4-way sorting is also possible for microscope slides. The sorter is equipped with an Automated Cell Deposition Unit (ACDU) module: A variety of multi-well formats can be used (e.g. up 384-well). *When sorting into plates, only 1 population at a time can be sorted into a well.*

ShadowFACS CONFIGURATION:

*** Scatter parameters are off the 488nm laser**

Laser Name	Detector	Filter	Dichroic Mirror	Examples of Fluorescent Labels that can be used
488nm 200mW	A	710/50 BP	670 LP	PerCP-Cy5.5, BB700
	B	530/40 BP	505 LP	FITC, AF488, GFP, YFP, Zombie-Green
640nm 120mW	A	780/60 BP	750LP	APC-Cy7, Zombie NIR
	B	720/40 BP	685LP	APC, AF647
	C	670/30 BP	-	Alexa Fluor 700, AF680, Draq7
532nm 150mW	A	780/60 BP	750 LP	PE-Cy7
	B	670/30 BP	650LP	PE-Cy5
	C	610/20 BP	600 LP	PE-TR, mCherry, 7-AAD, PI, PE-Dazzle
	D	580/30 BP	-	PE, tdTomato, AF594, RFP, TMRE, TMRM, PE-Dazzle
405nm 100mW	A	750 LP	740 LP	BV785
	B	610/20 BP	600 LP	BV605
	C	525/50 BP	495 LP	BV510, LD Aqua, CFP
	D	440/40 BP		DAPI, Pacific Blue, BV421, Hoechst, Zombie Violet, BFP, Dylight 405

HOW THE SORTING WORKS: *The Influx is a droplet generating cell sorter. These have a pair of high voltage plates and use electrostatic deflection to direct charged droplets (containing individual cells) into collection vessels. Droplets not marked for sorting will end up in the waste aspirator.* In a jet-in-air sorter, laser interrogation (where fluorescent labels are analyzed and populations of interest can be defined in the software for sorting) occurs after the sample stream has passed through the sorter's vibrating nozzle. This occurs relatively close to the point of exit from the nozzle, before the stream begins breaking off into saline droplets due to acoustic vibrations applied by a piezo device. At the time of formation, if a droplet was expected to contain an individual cell to be sorted, a charge is applied momentarily to the sample stream to produce a charged droplet (+ or -, up to 3 magnitudes for each to yield 6 possible collection streams). Non-sorting droplets are not charged. The droplets fall downward between a pair of high voltage plates in the sort chamber. Depending on their charge, they are either deflected left or right to a designated collection vessel or else continue their path directly falling into the waste aspirator.

SORTED VOLUME, AND SORT SPEEDS: Sort speed "in theory" can be estimated on the interplay of nozzle size, sheath tank pressure and number of droplets that can be generated (smaller nozzle + higher pressure = more drops). In practice, a big part of it is also the quality and nature of the sample biology (clumping, size, viability, type/"fragileness" and debris amount), which impacts the trade off on speed vs recovering cells with reasonable loss. Sort volume is linked to the nozzle size and sort mode we can use for your sort. The core has a wealth of experience working with difficult sample types. Contact fccf@salk.edu for help on optimizing your sort.