GW Flow Cytometry Core Facility

Updated June 2020

BD Influx Sorter

CONFIGURATION 4-LASER 15-COLOR	
Detector (Emission Range)	**Common Fluorochromes**
405 (100mW)	
425/26 (412-438)	AlexaFluor405, Pacific Blue, eFluor450, Sytox Blue, Zombie Violet, Calcien Violet, BV421, DAPI
520/35 (503-538)	AlexaFluor430, CFP, Live/Dead Aqua, Zombie Aqua, Live/Dead Yellow, AmCyan, BV510, Qdot525
610/20 (600-630)	BV570, BV605, Zombie Yellow, Pacific Orange, Qdot605
660/20 (650-670)	BV650, Qdot655
710/50 (685-735)	BV711 Qdot700
780/60 (750-810)	BV785, BV786, Qdot800
488 (100mW)	
530/40 (510-550)	AlexaFluor488, FITC, BB515, GFP, YFP, CFSE, Sytox Green, Zombie Green, Calcein AM, DyeCycle Green
692/40 (672-712)	PerCP, PerCPcy5.5, PerCPeFluor710
561 (150mW)	
585/29 (515-545)	PE,DSRed, tdTomato, Sytox Orange
610/20 (600-620)	AlexaFluor594, PE Dazzle594, ECD, mCherry, mKate, Zombie Red, PI
670/30 (655-685)	PEcy5, 7AAD
750LP (750)	PEcy7
640 (120mW)	
670/30 (655-685)	APC, AlexaFluor647, Sytox Red, e2-Crimson, Dye Cycle Ruby
720/40 (700-740)	AlexaFluor700, APC-R700, APCcy5.5
780/60 (750-810)	APCcy7, APC-H7, APCeFluor750, Zombie NIR

Tips for sucessful multicolor panels

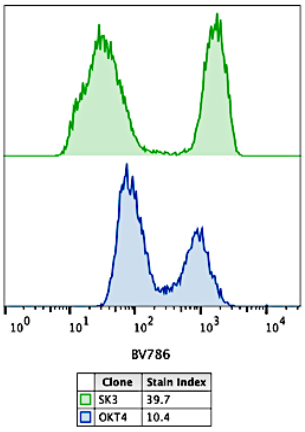
1. Know your gating strategy
2. Pair antigens to fluorophores optimally
   a. Based on expression level
      3 categories:
      -primary (well characterized, on/off expression): low stain index fluorophores
      -secondary (well characterized, continuum expression): low to medium stain index fluorophores
      -tertiary (low or unknown expression): high stain index fluorophores
   b. Based on fluorescence spreading
      Stain cells or compensation beads with ideal panel; use FlowJo/FCS Express to create a Spill Over Spread Matrix (SSM) to confirm spread is manageable for population identification
      *keep your gating strategy in mind. In the case of high spreading, use mutually exclusive/non co-expressed markers
3. Titrate your antibodies under your experimental conditions and calculate the Stain Index
   Stain Index formula:
   MFI (positive population)-MFI (negative population) / 2xrSD (negative population)
   ***MFI*= median fluorescence intensity**
   ***rSD*= robust standard deviation**
   Consider if you need to stain for separation or saturation
4. Make sure to use the appropriate controls *listed are a few examples*
   -Unstained cells: help with analyzing cellular autofluorescence
   -Compensation: single color stained cells or beads to correct spillover
   -Fluorescence Minus One (FMO): gating control based on autofluorescence and fluorescence spillover
   -Biological: treated vs non-treated, stimulated vs. non-stimulation, positive control

STAIN INDEX

Fluorochrome (RPA-T4 clone)	Relative Brightness
PE	8.0
PEcy5	7.5
Alexa Fluor 647	7.0
PE Dazzle594	6.5
BV421	6.0
Alexa Fluor 488	5.5
APC	5.0
PerCP eFluor 710	5.0
BV711	4.5
BV650	4.5
*BV786	4.5
BV605	4.0
PEcy7	4.0
PerCPcy5.5	3.5
FITC	3.5
BV785	3.0
BB515	2.5
BV510	2.5
Alexa Fluor 700	2.5
Pacific Blue	2.0
**BV786	2.0
APCcy7	1.5
BV570	1.5
APC-H7	1.0
APC Fire750	1.0

*SK3 clone
**OKT4 clone

Keep in mind this a general guideline and can vary depending on clone ex below: BV768 different clones



QUESTIONS?
We're here to help

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Additional Instrument Information

- ⇒ **BSL2+ sorting only**
- ⇒ Nozzle/Pressure: 100um/24psi (default), 70um/52psi, 85um/45psi, 140um/5psi, 200um/2psi
- ⇒ Sample tube: Falcon® 12x75mm round bottom **polypropylene** tube
- ⇒ Sample Collection Options: 5ml, 15ml, 50ml tubes, 1.5ml microcentrifuge tubes, 24,96,384 well plates