GW Flow Cytometry Core Facility

Updated August 2020

BD Celesta Analyzer w HTS

CONFIGURATION 3-LASER 12-COLOR	
Detector (Emission Range)	**Common Fluorochromes**
405 (50mW)	
450/40 N/A (430-470)	AlexaFluor405, Pacific Blue, eFluor450, Sytox Blue, Zombie Violet, Calcien Violet, BV421, DAPI
525/50 505LP (500-550)	AlexaFluor430, CFP, Live/Dead Aqua, Zombie Aqua, Live/Dead Yellow, AmCyan, BV510, Qdot525
610/20 595LP (600-630)	BV570, BV605, Zombie Yellow, Pacific Orange, Qdot605
670/30 655LP (655-685)	BV650, Qdot655
780/60 750LP (750-810)	BV711, BV785, BV786, Qdot800
488 (20mW)	
530/30 505LP (515-545)	AlexaFluor488, FITC, BB515, GFP, YFP, CFSE, Sytox Green, Zombie Green, Calcein AM, DyeCycle Green
575/25 550LP (563-588)	PE, PE Dazzle594, DSRed, Sytox Orange
695/40 670LP (675-715)	PerCPcy5.5, PerCPeFluor710, PEcy5, PI, 7AAD
780/60 750LP (750-810)	PEcy7
640 (40mW)	
670/30 N/A (655-685)	APC, AlexaFluor647, Sytox Red, e2-Crimson, Dye Cycle Ruby
730/45 690LP (708-753)	AlexaFluor700, APC-R700, APCcy5.5
780/60 750LP (750-810)	APCcy7, APC-H7, APCeFluor750, Zombie NIR

Tips for sucessful multicolor panels

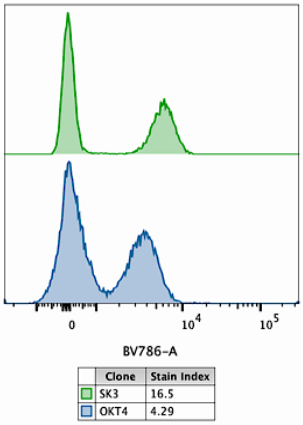
1. Know your gating strategy
2. Pair antigens to fluorophores optimally
   a. Based on expression level
      3 categories:
      -primary (well characterized, on/off expression): low stain index fluorophores
      -secondary (well characterized, continuum expression): low to medium stain index fluorophores
      -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
BV421	8.0
PE	7.0
Alexa Fluor 647	6.0
PEcy5	6.0
*BV786	5.5
APC	5.0
Alexa Fluor 488	5.0
BV510	4.5
PerCP eFluor 710	4.0
Pacific Blue	4.0
BV650	4.0
BV605	4.0
PE Dazzle 594	3.5
FITC	3.0
PerCPcy5.5	3.0
BV785	3.0
APCcy7	3.0
PEcy7	2.5
Alexa Fluor 700	2.5
BV711	2.0
BV570	2.0
APC Fire750	1.5
APC-H7	1.5
**BV786	1.0
BB515	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

Contact:
Kim Acklin, Manager
kjacklin@email.gwu.edu
202-994-0775

Additional Instrument Information

- ⇒ Sample tube mode: Falcon® 12x75mm, 5 mL **polystyrene** tube
- ⇒ High Throughput System (HTS) mode: 96 well plate-U, Flat, V bottom; 384 well plate-Flat bottom only
- ⇒ Software: BD FACSDIVA v 8.0.1.1