Guidelines for cell sorting

The CCRI FACS Core Unit is equipped with a BD FACSAria Fusion for cell sorting. FACS sorts are exclusively performed by our trained FACS operators. Sort slots need to be booked in advance (in person or via facs_core_unit@ccri.at).

Technical parameters of our BD FACSAria Fusion instrument:

- 4 laser lines (405nm, 488nm, 561nm, 640nm)
- 16 fluorescence channels / 18 parameter sorter
- 4-way sorting
- bulk and single cell sorting (tubes and multiwell plates)
- index sorting
- nozzle size: 70µm**,** (100 µm upon request)

Laser line and filter setup for panel design:

COLOR WORKSHEET ARIA FUSION

	405 nm laser						488 nm laser			561 nm laser				640 nm laser		
Filter	450/40	525/50	610/20	660/20	710/50	780/60	530/30	695/40	710/50	582/15	610/20	710/50	780/60	670/30	730/45	780/60
Colors	Pacific Blue	Pacific Orange					Fitc	PerCP	PerCP-eFl710	PE	PE-Texas Red	PE-Alexa 700	PE-Cy7	APC	APC-Cy5.5	APC-Cy7
	HorizonV 450	HorizonV500	Qdot 605	Qdot 655	Qdot 700				PerCP-Cy5.5		ECD		PC7	Cy5	APC-Alexa 700	APC-H7
	Syto 41	(AmCyan)					Syto 16				PE-CF594				APC-R700	APC-eFluor780
	eFluor450	Krome Orange	eFluor605NC	eFluor650NC										eFluor660		
	Alexa 405	Alexa 430					Alexa 488			TRITC	PE-Alexa 610			Alexa 647	Alexa 680	APC-Alexa750
	CFP						GFP/YFP			DsRed	mPlum/mCherry				Alexa 700	
	DAPI/Hoechst	BV480	(PJ)				CFSE	PJ/ 7AAD	PJ	PJ	PJ			Topro3		
	BV 421	BV 510	BV 605	BV 650	BV 711	BV 786	BB515		BB700		PE-Dazzle594					APC-Fire750
	VioBlue	VioGreen					Vio 515		PerCP-Vio700		PE-Vio 615		PE-Vio 770	Vio667		APC-Vio 770

Please consult with the staff of the CCRI FACS Core Unit before designing new sort experiments for the best choice of fluorophores and necessary control samples to establish correct instrument settings.

Important considerations

1) Biosafety

Flow cytometry inevitably generates aerosols. Whenever using lentiviral/retroviral transduction procedures, please adhere to biosafety rules as outlined in the document "K34-rules".

- Transduced cells will only be sorted after 7 days of culture with at least 3x media exchanges.
- For special circumstances, please contact the FACS Core Unit and manfred.lehner@ccri.at.

2) Cell numbers for sorting

What you should know beforehand are i) the desired number of sorted target cells and ii) the percentage of the target cell population in the starting material.

Example:

- desired number of sorted target cells = 10,000
- percentage in starting material = 1%
- $1x10^6$ cells + at least 20% extra (considering sorting abort rate and aggregates) = $1.2x10^6$ in total needed
- keep in mind that each washing step can lead to 10% cell loss

3) Health of starting population

During FACS sorting cells are exposed to various stresses and forces, including high pressure, shear forces, laser illumination and temperature changes. This can lead to high cell death and compromised functionality post sort. The healthier your starting population is, the better it will withstand sorting-induced stress. Generally, adding BSA or FCS to your buffer will help keep your cells in better shape pre -and post-sort.

4) Cell concentration and volume for sorting

- maximum cell concentration: $2.5x10^7$/ml
- minimal sample volume: 250µL

5) Suitable buffers

- PBS, tissue culture media with a maximum of 2% FCS

6) Adherent cells

- Cells should be detached with Accutase or Accumax to achieve a high-quality single cell suspension.
- Please test beforehand the impact of cell dissociation reagents on the surface expression of your markers of interest.

7) Sample filtering

- To avoid instrument blockage during sorting, you need to filter your sample shortly before bringing it to the FACS Core Unit. We recommend Falcon 352235 ("5ml Polystyrene Round-Bottom Tube with Cell-Strainer Cap")
- Besides 5ml "FACS tubes", cells can also be acquired from Micronic tubes and 15ml centrifuge tubes

8) Target cell collection

- four (4) populations can be sorted simultaneously into microcentrifuge tubes and 5ml "FACS tubes"; only two (2) populations for 15ml centrifuge tubes
- one (1) population or single cells per well can be sorted into multiwell plates (6, 24, 48, 96, 384 well)

- some cells stick to polystyrene tubes, so you might want to use polypropylene tubes to minimize cell loss
- rinsing your collection tube with FCS can reduce cell adhesion
- always sort buffer or tissue culture media. Avoid sorting into dry tubes
- sorted cells take up volume:
    - 1000 cells ~ 1µl
    - $1x10^6$ cells ~ 1ml
- up to 25,000 events per second ($90x10^6$ per hour) can be processed
- Please make sure to add antibiotics if you want to cultivate sorted cells further. Penicillin/Streptomycin should suffice. Normocin (InvivoGen) can in addition protect against fungi and mycoplasma.