Flow Cytometry Lab A214

INSTRUMENT INFORMATION:

Beckman Coulter MoFlo XDP Cell sorter is equipped with a 488nm Argon laser (Filters:529/28;625/26;575/25;670/30;785/62) and 633nm HeNe laser (670/30;785/62) and is located in the biosafety level-2 (BSL-2) laboratory space in VIDO A214. Additional filter set up is available.

Single cell suspensions can be characterized and sorted at 10-30,000 events per second based on two light scattering parameters (FSC, SSC) and up to seven fluorochrome colours.

Up to four distinct cell populations can be sorted simultaneously from a single cell suspension. Cell populations can be sorted on the basis of either the presence or absence of defined surface molecules or other physical cell characteristics.

SAMPLE REQUIREMENTS:

- Cells must be a single cell suspension. Cell clumps can clog the machine.

- Optimal cell concentration in a sample tube is 50-100 million cells/ml. This is important since cell concentration influences speed of sorting.

- Minimal volume per tube is 500ul and recommended tubes are 5 ml polystyrene tubes (available in Lab 214 on request)

- Cell suspension should be re-suspended in calcium and magnesium free PBS (pH 7.2; GMP) and filtered through a 40 µm mesh (Cell strainer; BD Falcon Cat# 352235) immediately prior to sorting.

- Unstained cells, isotype control samples, and single color fluorochrome labelled samples $(1 \times 10^6 \text{ cells})$ per sample) are required for optimum MoFlo set-up and defining sort gates.

- FACS profiles are required for all target cell populations prior to booking a sort. This enables the MoFlo specialist to ensure cell labeling is effective and is necessary when setting sort gates for the cell sort.

FEES FOR CELL SORTING:

- MoFlo must be booked a minimum of one week prior to use by contacting Dr. Philip Griebel (philip.griebel@usask.ca; Tel: 1542) or Natasa Arsic (Natasa.Arsic@usask.ca; Tel: 1556 or 1531).

- Users who cancel a sort less than 24 hours prior to scheduled time or fail to show on the reserved date will be charged for all scheduled sort time.

- Users who cancel a scheduled sort less than 7 days in advance will be charged a \$50 cancellation fee unless the sort time is booked by another user.

- The daily sorting fee is \$350 which includes machine set-up and shut-down after the sort, and all consumables (sheath fluid, tubes, etc.).

- Users who require sort time beyond 5:00 pm will be charged an additional \$100/hour, including time to shut down the MoFlo.

- If a sort cannot be completed due to a MoFlo failure (software or hardware) there will be no charge for the sort.

- Issues regarding billing or MoFlo access will be resolved in consultation with Dr. Philip Griebel.

NOTE:

Cells will be sorted into polystyrene tubes coated with 4% BSA and 200ul of FBS will be added to each tube prior to sorting. This prevents damage of sorted cells and optimizes cell viability. Alternative conditions can be used if necessary for experimental purposes.

MoFlo XDP – Cell Sorting Service Request Form CLIENT INFORMATION:

Name:				
Position:	PI	Post Doctoral Fellow	Research Technician	Graduate student
College/Inst	titution	:		
Billing Add	ress:			
Account Nu	mber:			
Phone Num	ber:			
E-mail add	ress:			
Principal Inv SAMPLE I	vestigat	or:(Signature)		
Species:				
Tissue:				
Total Viable	e Cells/	Sample:		
Sample Vol	ume: _			
Does sample	e conta	in a pathogen: No	Yes: Pathogen:	
Transfected	Cells:	No Yes :T	ransfection agent:	

SORTED CELL REQUIREMENTS:

	%	Sorted	% Purity
Target Cell Phenotype	Target Cells in	Cell Number	Required for
(Surface markers)	Unsorted Sample	Required	Sorted Cells

SAMPLE LABELING INFORMATION:

Cell Surface Molecule	Primary Antibody and Concentration	Secondary Reagent and Concentration	Fluorochrome

The information above must be reviewed with a MoFlo Technical Specialist <u>prior to scheduling a</u> <u>sort.</u> This will ensure the MoFlo is an appropriate tool to provide the number and purity of cells required and that cell labeling is optimized for MoFlo sorting. This will also ensure that samples for sorting can be provided in a timely manner to ensure sorting is completed during normal working hours.

FOR INTERNAL USE ONLY

INSTRUMENT SETUP:

Date:
Technical Specialist:
Frequency:
Amplitude:
Charge Phase:
Defanning:
Plate Voltage:
Drop Delay:
EPS Range:
Hard Abort Rate Range:
Data File Storage:
O.T

TECHNICAL OBSERVATIONS:

RECOMMENDATIONS: