The Flow Cytometry Facility

University of Glasgow

SAMPLE PREPARATION FOR FACS ANALYSIS

Whatever your cell type - samples must be a good quality high viability single cell suspension.

SAMPLE TUBES

All samples must be loaded onto the CALIBUR and LSRII in 12x7.5ml BD FACS tubes (BD cat number BD2058). These are available from MVLS Stores.
The same tubes are compatible with the MACS Quant. Additionally the Quant can also run 1.5ml eppendorf tubes, and 96 well plates.

The CyAn ADP uses blue Beckmann Coulter tubes cat no: 2523749 (available from the FCF or MVLS stores). The machine aspirates your entire sample to waste if you do not use Beckmann tubes.

TEMPERATURE

Keep your samples chilled on ice, as this will prevent formation of aggregates, unless your protocol will not allow this. Keep your samples in the dark.

SINGLE CELLS

You MUST filter your samples just before running them through the machine - use:
Nitex nylon mesh
or BD falcon cell strainers (40-70 micron)
or BD FACS tubes with cell strainer cap (ref 352235)
or Cell Trics, which are available from Partec UK.

FACS BUFFER – basic recipe

For best results use PBS supplemented with 1% dialysed FBS. Using the lowest possible concentration of protein will reduce auto fluorescence.

If your cells have a tendency to clump use Ca/Mg ++ free PBS.
Add EDTA (1mM-5mM) to the buffer to prevent formation of aggregates.

Add 25-50ug/ml of DNAse I and 5mM magnesium chloride hexahydrate if cells are clumping due to cell death.

LIVE /DEAD DISCRIMINATION

Always use a viability dye to exclude dead cells.
When staining cells with conventional dead cell exclusion dyes, e.g PI, 7-AAD, Hoechst, DAPI, add just before running your stained sample.
After 5 minutes incubation wash the viability stain off before running your sort sample. (NB these stains cannot be fixed)
There is a good range of new viability dyes available for many fluorescence channels, which are also fixable. e.g Live /dead aqua, eFluor 450, and eFluor780. Stain the cells with these viability dyes *before* surface staining. Care must be taken to use the appropriate buffer. Follow the instruction on the data sheet.

SAMPLE CONCENTRATION

The sample concentration for FACS analysis should be around 1 x 10^6cells/ml depending on the cell type.

COMPENSATION CONTROLS

Please provide all the necessary controls in order for your experiment to be valid

Negative Control: 0.5 to 1x 10^6 cells/ml non-stained cells.

Single stained compensation controls: Provide 0.5 to 1x 10^5 cells/ml of single stained cells for every colour you are using.
Include negative cells in each single colour control tube.

If you intend to use comp beads to set up the compensation bring:
Unstained Cells: 0.5 to 1x 10^6 cells/ml.
Unstained beads
Single stained beads for each colour, using the same antibodies you will use to stain your cells.

FLUORESCENCE MINUS ONE GATING CONTROLS

If antigen expression is low, or differential within a population, then FMO controls can be used to set gates for positive cells.
FMO control tubes are stained up with all the antibodies in your panel minus one

CONTROLS FOR TRANSFECTED CELLS

Please bring mock-transfected cells (no FP expressed)
If your sample cells are going to express several fluorescent proteins simultaneously, please bring along the control cell lines for each fluorescent protein you will be using, which express a only a single Fluorescent protein.

FIXING CELLS

If cells are being fixed for safety, rather than convenience fix them in 4% Formaldehyde for 24hrs before FACS analysis.
They should then be washed twice and resuspended in FACS buffer.
Be aware that fixing your cells will lead to increased auto fluorescence and changes in FSC/SSC.

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