SOP for analysing/sorting adherent cell lines

FACS buffer (x10 to make a concentrate)
1x PBS (human or mouse depending on cells)
2-5 % (v/v) FBS or BSA (ABS for human cells)
0.5 mM EDTA (increase to 2mM for sticky or adherent cell lines)
2 mM NaN3

Pass through a 0.2um filter to sterilise and remove any FBS or BSA debris

Note: NaN3 is added as a preservative. Use the buffer without NaN3 if you want to do functional assays with bacterial cells.

Preparation of cells stored in liquid nitrogen

This method provides a general procedure for use when using cells from liquid nitrogen.

Prepare FACS buffer
Carefully remove cells from liquid nitrogen storage.
Thaw cells rapidly in a 37^{o}C water bath.
Resuspend cells in cold FACS buffer by slow drop by drop method and transfer them to a 15 ml conical centrifuge tube.
Centrifuge at 300-400 g for 5 min at 4^{o}C.
Resuspend in known volume of FACS buffer and do cell count.
Discard supernatant and resuspend 1x10^6 cells/ml for analysis and 1x10^7 for sorting in cold (4^{o}C) FACS Buffer
NB: higher viability can be obtained by allowing the cells to recover in culture media overnight.

Preparation of tissue culture cell lines in suspension

This method provides a general procedure for use with tissue culture cells in suspension.

Prepare FACS buffer.
Gently pipet cell suspension up and down in the culture flask and then transfer into 15ml conical centrifuge tube(s).
Centrifuge at 300-400 g for 5 minutes at room temperature.
Discard supernatant and resuspend pellet in 10 ml of room temperature FACS buffer.
Centrifuge at 300-400 g for 5 minutes at room temperature.
Resuspend in known volume of FACS buffer and do cell count.
Discard supernatant and resuspend 1x10^6 cells/ml for analysis and 1x10^7 for sorting in cold (4^{o}C) FACS Buffer

Preparation of adherent tissue culture cell lines

This method provides a general procedure for use with adherent tissue culture cells

Harvest cells by enzymatic release using 1x Accutase solution or 0.25% trypsin, followed by quenching with media containing serum.
(**Note:** epitopes may be cleaved when using the enzymatic digestion method. Cells can also be harvested by gently scraping them into culture media.
Prepare FACS Buffer
Remove the culture medium and eliminate residual serum by rinsing cell monolayers with sterile, room temperature PBS.
Slowly add 1x Accutase solution or 0.25% Trypsin to cover the cell monolayer.
Incubate at 37^{o}C for up to 10 minutes.
After incubation gently tap the flask and the cells will detach and slide off in one sheet to the bottom of the flask.
Add growth medium and re-suspend the cells by gently pipetting.
Centrifuge at 300-400 g for 5 min.
Discard supernatant and resuspend pellet in fresh, room temperature PBS/BSA to wash off any remaining cell debris and proteins.
Centrifuge at 300-400 g for 5 minutes at room temperature.
Discard supernatant and resuspend pellet in an appropriate amount of room temperature PBS/BSA.
Count cells using a hemocytometer or an automated cell counter
Once counted dilute the cells with cold (4^{o}C) PBS/BSA to a minimum concentration of 1 x 10^{7} cells/ml.