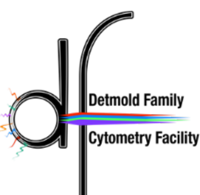


Recommended controls

You need to include adequate controls for your experiment to have confidence in drawing conclusions from, and interpreting the data.

Consequences:

Conclusions or hypothesis founded on false positive/negative data.

The data acquired cannot be used for publication – so, you need to repeat the experiment.

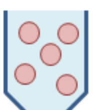
Single colour experiments

Controls for

Unstained cells

Setting voltage of your fluorophore (auto-fluorescence)
Shows where negative cells reside in your data
Setting FSC and SSC of your cell type

Isotype control (antibody-based flow)



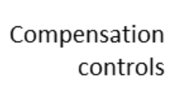
Non-specific binding of your fluorescent-antibody

Experimental control (e.g. ± compound)

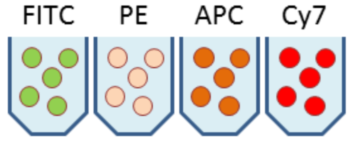
Cell population FSC and SSC changes caused by experiment
Changes in auto-fluorescence

Multi-colour experiments

All of the controls mentioned above, plus:



Compensation controls



Single-stained tubes for every fluorophore in your assay
Needs to be performed EVERY; yes, EVERY flow acquisition

Controls for
Spectral overlap of your fluorophores into channels other than its own
Visualise spectral spread after applying compensation

FMO controls

	FITC	PE	APC	Cy7
FMO 1	-			
FMO 2		-		
FMO 3			-	
FMO 4				-

A series of controls with all but one of your colours over every fluorophore (see table)

Mandatory for every colour the first time you run your assay.

You may not need to run a FMO for every colour on day-to-day runs :D

Controls for
De-convolutes negative and positive populations within a complex colour assay better than single-stain or isotype controls.
See Detmold staff, or flow-literature for a through explanation.