RECOMMENDED CONTROLS FOR FLOW CYTOMETRY / FACS

Along with the samples to be labelled, the following controls should be used whenever possible. The various positive controls are used for compensating and gating when setting up the flow cytometer.

Control	Sample type	Primary Ab	Secondary	Reason
Cells only Use treated and untreated cells	Neg control cells	No	No	Negative Control/Background Autofluoresence control
Primary Ab control	Neg control cells	Yes	No	Check for non specific binding of primary
Treated primary Ab control	Treated cells	No	Yes	Check for non specific binding of secondary Ab on treated cells
Isotype control	Neg control cells	Use Isotype control antibody. This should be the same antibody isotype as primary antibody. *	Yes	To confirm that the primary antibody binding is specific and not a results of non-specific Fc receptor binding or other protein interactions.
Compensation controls for each fluorochrome	Positive population of labelled beads or positive control cell sample	Yes	Yes	Positive control to set up cytometer alignment and to remove spectral overlap.
Cell viability control	Cell sample (identical to other samples) stained with both antibody and PI nuclear stain	Yes	Yes	Nonviable cells can be discriminated from live cells on the basis of light scatter (FSC=forward scatter). This discrimination is often lost in fixed or permeabilized cells. In these cases dead cells can be distinguished from live cells by their uptake of fluorescent DNA dyes due to loss of membrane integrity e.g. PI (propidium iodide) is used for dead-cell discrimination in unfixed and non permeabilized cells. 7-AAD (7-aminoactinomycin D, fluorescent) + AD (actinmycin D, nonfluorescent) for fixed or permeabilized cells.
Specificity control	Cell samples	Yes. With excess non-labelled primary.	For direct staining only	Add excess unlabelled primary antibody with normal amount of labelled primary. If staining is specific, the non-labelled primary should compete with labelled primary and reduce the fluorescence observed.
Treated secondary Ab control	Treated cells	No	Yes	Check for non specific binding of secondary Ab on treated cells

*Isotype controls can also be raised against an antigen known not to be present in the sample, eg KLH.