Instrument Set Up & Analysis Control

Flow Cytometry Unit

Control and application stage	Notes
UNSTAINED CONTROL Include: Unstained sample with no antibody ideally in every experiment	Control for background autofluorescence used to determine positivity • Consider auto fluorescence profile of the sample when choosing flurochromes. • Try using a different excitation laser or detector if autofluorescence is high .
COMPENSATION CONTROL Include: Single stained tubes for each color in the panel ideally in every experiment (attention to tandem lot and uncoupling)	Compensate spillover in multicolor panels where fluorescence overlap between flurochromes is expected (use antibody capture beads or cells) • Use same fluorophore or dye than the sample. In tandems same lot must be used. • Controls must follow the same protocol than the sample (fixation and permeabilization buffers) • Controls must have a positive and negative population with identical autofluorescence • Positive population must be as bright or brighter than in the sample • Collect enough number of events to be statistically significant

Staining Control

Control purpose and application stage	Notes
VIABILITY CONTROL *Staining Control (No Specific Binding): Dead Cells Control* Include: DNA based dyes or amine reactive dyes ideally in every experiment	Remove staining artifacts and no specific binding (NSB) from highly autofluorescent dead cells • Use DNA cell impermeant dyes for nucleic acid stain in cells with compromised membranes • Use anime reactive dyes (fixable dyes) for fixed cells or to increase detection options.
REAGENT CONTROL *Staining Control (No Specific Binding, NSB): Electrostatic Interactions* Include: antibody titrations at characterization stage or new lot introduced	Reduce NSB due to electrostatic interactions • Too high concentrations cause more non-specific binding • Especially important in intracellular antigen staining
FC BLOCKING CONTROL *Staining Control (No Specific Binding): Surface Fc receptors* Include: FC blocking reagents in protocol prior to staining at characterization stage/ in every experiment	Avoid increased background and false positives due to Ab binding though cell surface Fc receptors • Saturate Fc receptor with specific Abs (FcBlock CD16/32) or preincubate with the serum of the host of the primary antibody • Use of commercially available Fab fragment (REAfinity) antibodies
ISOTYPE CONTROL *Staining Control (No Specific Binding)* Include: cells labeled with abs with irrelevant specificity (same isotype and flurochromes) at characterization stage	Assess the effectiveness of the Fc blocking protocol • The use of isotype controls for gating purposes is not recommended (antibody and isotypes may show different affinities and different F:P ratios)
SECONDARY ANTIBODY CONTROL *Staining Control (No Specific Binding)* Include: Cells stained only with secondary Ab at characterization stage or new lot introduced	Evaluate nonspecific binding of the secondary antibody • Only for samples with indirect staining with secondary antibody or streptavidin

Analysis Control

Control and application stage	Notes
FMO CONTROLS *Analysis Control* Include: cells stained with all flurochromes minus one for each color in the panel in every experiment	Detect fluorescence spread in a particular detector introduced by the rest of the flurochromes in the panel. Aids in gating placement. • Especially important in the case of dimly expressed antigens or when antigen expression is unknown. • Also in detectors where fluorescence spillover from bright flurochromes is expected.