Sample Preparation Guidelines

1) SAMPLE

a. The preparation should be a single cell suspension; this can be achieved by filtering with a 30 to 90µm mesh filter, being 30µm the most efficient and commonly used.
b. Cells can be suspended in PBS/1%BSA, PBS/2mM EDTA or culture medium.
c. The sample volume must be at least 500µl.
d. A minimum of $2x10^5$ cells per sample is required for basic analysis
e. Cell concentration should be between 10^6 and $20x10^6$ cells per ml for sorting.
f. The sample should be in a **5ml round-bottom polypropylene tube.** (ex: Falcon 352063).
g. Additional buffer needs to be brought in case the sample to sort requires dilution.

2) COLLECTION

a. Cells can be collected in a variety of vessel and well-plate sizes. The MoFlo XDP can sort cells into 1.5ml microtubes, 5ml round-bottom tubes, 15 or 50ml conical tubes and well-plates from 6 to 384 wells. For 4-way sorting (MoFlo XDP), cells are collected in 1.5 ml tubes or 5ml round-bottom tubes.
b. The sorted cells can be collected in PBS or culture medium. The collection liquid should contain a buffer, and the presence of serum is known to improve the viability of sorted cells.
c. The collection tubes should be filled to one fifth of total volume with the collection liquid. Please note that the collected sample will be diluted with PBS from the instrument's sheath fluid.

3) CONTROLS

Please discuss the necessary controls for your experiment with the instrument operator.

a. Negative controls

1. **Autofluorescence control** (for detection of autofluorescence background): Consists of cells without fluorescence. This control is mandatory.
2. **Secondary control** (for detection of non-specific binding of secondary antibody): Consists of cells with the labeled secondary antibody in the absence of primary antibody.

b. Positive controls (Each positive control should have a single fluorescence parameter)

1. **Compensation controls** (to eliminate overlap of fluorescent molecules): Consists of cells with each individual type of fluorescence used (labeled antibody or fluorescent molecule).
2. **Isotype control** (for detection of non-specific binding of primary antibody): Consists of cells with a labeled "irrelevant" antibody of the same isotype class as the reagent.