Procedure

1. Perform cell staining as indicated in the protocol of immunofluorescence staining: http://gscf.iss.it/dw/lib/exe/fetch.php?media=aree:citometria:documenti:immunofluorescence_staining.pdf

2. Resuspend cell pellet in PBS w/ 0.1% BSA at concentration of 10^7 cells/ml. The cells need to be filtered, through 70µM filter, to prevent clogging.

3. Add Propidium Iodide or other available viability dye to unfixed stained cells to discriminate live from dead cells.

4. An unstained sample or negative Ig control and single stained samples for each conjugated dye are also necessary for setting up the flow cytometer. Refer to the below guidelines for the specific control: http://gscf.iss.it/dw/lib/exe/fetch.php?media=aree:citometria:documenti:immunofluorescence_staining.pdf

COLLECTION TUBES

Cells cannot be sorted in empty tubes, therefore provide tubes (to collect purified cells) filled with the specific media.

Sample may be collected using different devices:

- 2 way sorting: cells are sorted into 15ml tubes.
- 4 way sorting: cells are sorted into 5ml FACS tubes

Sorting into multiwell plates and/or microscope slides is also possible.

Recommendations:

Polypropylene tubes are better than those of polystyrene: electrostatic charges from polystyrene tubes can provoke sticking of cells to the side of the tubes, favouring cells loss due to adherence of charged drops onto plastic and then reducing sorting efficiency. In order to prevent this phenomenon, we suggest to coat the tubes filling them with 4% BSA for at least 1 hour before the sorting or overnight at 4ºC.

The tubes should be contain media to keep cells vital: for example, for 15 ml tubes, fill them with 3ml media.

COLLECTION MEDIA

Should be optimized for your cells: as an example, you can use PBS supplemented with 10-20% FCS or culture media.

CELL RECOVERY

The rate of cells sorted is always less than the expected because of **several factors** (threshold rate, flow rate, sample quality, target cell frequency sort aborts or cells sticking to the collection tubes) that could affect the sorting procedure. Therefore, it is reasonable that the sort yield is about 50% of the cells counted by FACS (theoretical yield).

For low frequency or for rare cells populations it is recommended to enrich the sample for the population of interest, with the advantages of a faster sorting and higher yield of cells more viable and pure.