How to prepare cell samples for cell-sorting

The cells:

To get the maximum yield and purity the scientist needs to prepare viable cells out off liquids or organs. Cell lines must be harvested in the log-phase, adherent cells have to be detached from the surface gently.

Make sure to have single cell suspensions!

The staining:

Using a stream in air cell-sorter means a loss of fluorescence sensitivity compared to an analyzer.

Make sure to have antibodies optimized and titrated for the experiment.

Remember the good dyeing practices. Have the cells cold and dark whenever it is possible.

Never use fading fluorochromes. Try to minimize spectral overflow.

Bring controls like isotypes and unstained cells. For transfected cell lines prepare controls out of untransfected but identically treated cells.

How many cells do you have to bring to reach your aims?

This depends on the preparation. You have to imagine that not all of the cells one is counting is suitable for successful cell-sorting. One might loose up to 40% already by gating on viable singlets. Sort aborts caused by high purity modes also give rise to cell losses. Depending on the desired sorting speed this could bring down the yield about 30%.After all the cells are hardly strained by the treatment in the machine. This could lead to a loss of further 20-40%.

What you have to bring with you.

Bring single cells in suitable medium at a cell number of about $5x10^7$ per ml.

Bring the tubes or plates the cells have to be sorted in.

It is possible to sort up to 4 populations at a time into Eppendorf caps or 5ml FACS tubes.2 populations can be sorted in 15ml tubes. Cells can be sorted in every kind of cell culture or other plates. Different cell numbers and populations per well.

Further bring suitable medium.

Important!

To protect microbiological contamination always wash the cells with low centrifugation speed after sorting. Use antibiotics in the medium.