## Sample Preparation

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**Preparation of a single cell suspension:**

Samples for Flow Cytometry should be prepared as a single cell suspension. Concentration of cells can vary from 10e4-10e7. The most common suspension buffer is Phosphate buffered saline (PBS).

Cells, which are already present in a single form like non-adherent cells from tissue culture or blood cells, are simple to prepare. They are just collected, washed, stained and filtered before reading.

Cells from adherent cultures or solid tissues must be disaggregated in order to produce single cells. This can be done mechanically, enzymatically or by using chelators:

1. Mechanical disaggregation is suitable for loosely bound structures like adherent cells from culture, bone marrow and lymphoid tissue. It involves passing a suspension of chopped tissue through a fine-gauge needle several times, followed by grinding and sonication as necessary.
2. Enzymes are used to disrupt protein-protein interactions and the extracellular matrix that hold cells together. Their action is dependent on factors including pH, temperature and co-factors so care must be taken when choosing an enzyme. Some treatments may damage cells if left unneutralized for too long and cell surface antigens of interest may be lost.
3. Chelators like EDTA and EGTA can remove divalent cations responsible for maintaining cell function and integrity but their presence may inhibit certain enzymes.

To study intracellular components e.g. cytokines by Flow Cytometry, the plasma membrane of the cell often has to be permeabilized to allow dyes or antibody molecules through while retaining the cell's overall integrity. The method of choice may involve a combination of permeabilization and fixation agents.

Note, the preferred method of disaggregation as well as permeabilization and fixation is often a trial-and-error process.

#### MEDIA FOR FLOW SORTING

1. Cells to be sorted are suspended in Calcium and Magnesium-free PBS. Serum can be added up to 1%.
2. Sorting media is also Calcium and Magnesium-free PBS. For experiments requiring culture of or functional analyses on sorted cells the PBS must be sterile.
3. Cells are sorted into tubes containing 0.5ml or more of pure serum.

#### FILTERING THE SAMPLES:

It is essential to filter all samples before reading them in a Flow Cytometer

During processing cells will pass through a 50 ֲµm nozzle tip on most instruments. Clumps and debris cann clog the instrument fluidics and either distort the measurements or obstruct them completely.

It is essential to filter cells through a 40-50 ֲµm nylon mesh after all staining proccedures. This should be carried out right before sorting.

#### FILTRATION WITH A REUSABLE SYRINGE, EXTENSION TUBE AND 50 ֲΜM NYLON MESH:A

Preparation: use syringe, extension tube and nylon mesh. Remove the needle from the syringe, locate the nylon mash on the edge of the syringe and connect the right side of the extension tube to the syringe with the mesh in-between them. Cut the extension tube about 1 cm from the connector.

Filtering: take out the piston rod, pour out the sample from the tube into the syringe and bring back the piston rod to the syringe push gently the piston into a new empty and clean tube. Wash the syringe filter with PBS between each sample.

Syringe: Bactlab (Becton Dickinson), various sizes.
Extension Tube: Teva Medical, Cat. No: MG918420. ?
Nylon Mash: A.D. Sinun, Cat. No: r0050h380315, 50 microns.

#### FILTRATION WITH A READY-MADE FILTER:

Use a pipette to force the cells through the filter into the tube.

**Filters:**

1. Partec GmbH, for sterile-Cat. No 04-004-2327, single packed 50um filters, for non sterile- Cat. No 04-0042-2327. Both are suitable for 5ml FACS tubes

Bactlab, Cat. No. FAL352340, sterile 40um filters - suitable for 50 ml tubes