Single Cell Sorting

UWCCC Flow Cytometry Laboratory
http://www.uwhealth.org/flowlab

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Biological Safety

Cell sorting creates aerosols that may pose a risk to the operator and others in the sort lab. Cell sorting must be included in your lab's Biological Safety Protocol and on file with the UW Office of Biological Safety.

Reserving the Sorter

Reserve a cell sorter 1-2 weeks in advance via the iLab scheduling system.

Sorting 3 96-Well plates for one sample takes 30-45 minutes per sample, depending on transfection efficiency. Add 30 minutes for experimental set up on the instrument. In the appointment request form, select the 130um/14psi or 100µm/20psi option for Nozzle Tip and Pressure and 96-Well Plate for collection device.

Cell Preparation

Prepare a single cell suspension. Cell concentration should be 5-10 million per mL. If there are fewer than 5 million total cells per sample, resuspend in no less than 0.5 mL.

Resuspension buffer should protect the cells from pH changes and aggregation. Suggested components include PBS free of Ca^{++} and Mg^{++}, 2% serum or BSA, 2-5mM EDTA, and 10-25mM HEPES.

Prepare 96-Well plates for collection by adding 200 µL media per well.

Immediately Prior to Cell Sorting

In the Flow Lab, filter your samples to be sorted in the biosafety cabinet. Flow Lab provides sterile cell filters, tubes, and pipets.

Immediately After Cell Sorting

Centrifuge plates for 30-60 seconds at 1200 RPM (300 x g) to settle the cells in the wells. The Flow Lab has an incubator available to store plates until all plates are completed.

Materials

Test tubes containing cells for sorting:

- 12x75mm 5mL or
- 15 mL conical

96-Well Plates for collection
Media

Provided by Flow Lab:

- Cell Filters
- Sterile Tubes for filtering
- Pipets for filtering
- Biological Safety Cabinet
- Incubator
- Centrifuge for 96-Well Plates

FAQ

Why do I have to filter my cells?
Filtering cells reduces cell aggregates in your sample to be sorted and reduces the chance of clogging the nozzle tip, resulting in potential contamination of collection device and loss of sample.

Why spin the plates after sort?
Sometimes the cell is deposited on the edge of the meniscus of the media and the edge of the well. A quick spin will help the cells settle in the wells and increase the incidence of viable colonies.