Sorting for Genomics FAQ

**How can I facilitate optimal recovery of sorted cells in my collection tubes/plate?**

Collection tubes/sample wells can be pre-coated overnight with high serum media or 100% FBS serum at 4 degrees Celsius in the fridge. Prior to the sort, decant the serum and replace with your desired volume of collection media. The serum acts to coat the sides of the tube so that sorted cells don’t impact a dry surface and also helps to negate any electrostatic charge in the collection vessels that could affect the path of the sorted droplets. For details on collection buffer, see below:

* Collection buffer for collecting cells post-sorting
  + Pre-coat the collection tubes by filling with one of the following options:
    - * PBS + 20% FBS
      * 100% FBS
    - Let filled collection tubes incubate overnight in the fridge at 4 degrees Celsius.
    - Immediately prior to sort, decant and add an appropriate volume of collection buffer to collection tube. Aim for ~10% final FBS at the end of the sort in your collection tube. Here are some rough guides:
    - PBS + 10% FBS for collecting <10,000 sorted cells
    - PBS + 20% FBS for collecting <500,000 sorted cells
    - 100% FBS for collecting >500,000 sorted cells

Make sure that cell counts are checked manually or with a cell counter. Sorted counts need to be verified for downstream assays.

**What Nozzle size should I use to sort nuclei?**

Sorting of nuclei is possible using the 70um, 85um or 100um nozzles. If nuclear integrity post-sort becomes an issue when sorting on the 70um, using the 85um-100um nozzles are advisable due to their lower operating pressures (~45psi and ~20psi, respectively) than the 70um (~70psi).

**What if there is a lot of debris in my sample?**

The dissociation protocol needs to be optimized. Trying to sort from a sample with a lot of debris only decreases sort efficiency and increases the time required to sort. There are a variety of cleanup strategies available. Here are some suggestions specifically using the Miltenyi MACs system:

* Debris removal solution:
  + <https://www.miltenyibiotec.com/IN-en/products/debris-removal-solution.html#130-109-398>
* Myelin removal (when dealing with brain and neuronal tissue):
  + <https://www.miltenyibiotec.com/UN-en/products/myelin-removal-beads-ii-human-mouse-rat.html#size=for-up-to-400-separations>
* Anti-nucleus microbeads (if isolating nuclei):
  + <https://www.miltenyibiotec.com/IN-en/au-en/products/anti-nucleus-microbeads.html>
    - The core has a magnet and magnet stand to use for this protocol.