DNAse I Treatment of cell samples for Flow Cytometry Version 1

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Free DNA liberated by dying cells is the most common issue affecting sample quality for flow cytometry. It is sticky, and viscous in high concentrations and can contribute to clogging issues when run on the cytometer. DNA can also accumulate on the flow cell, impacting the quality of the data being collected (in extreme cases, data will be nearly unrecognizable). For cell sorting, DNA mediated stickiness can additionally contribute to poor sort purity as it promotes attachment between cells (i.e. sort target cells and non sort target cells). Sorting samples have come to the core visibly viscous (and thus cannot be sorted) until treated with DNAse I.

The DNAse I treatment is highly effective as a standalone step, but can also be combined with multiple steps of sample prep as needed (e.g. cells being prepared from lamina propria, vs preparing a relatively clean spleen or lymphoid prep). Here are a few examples of where DNAse I treatment is commonly included:

- **During cell harvesting (depends on method/enzyme used)**
- **After cell harvesting before staining/labeling**
- **During fluorescence labeling and/or during wash steps (conduct a small pilot test when combining with labeling to be safe!)**
- **After final wash before fixing cells**
- **In FACS buffer used to resuspend cells for analysis (only if MgCl2 can be included).**

We recommend the inclusion of a DNAse I treatment step when working with whole cells if possible, however it requires the addition of $MgCl_2$ for catalytic activity (stabilizes the enzyme conformation). Please contact the fccf@salk.edu if you are running nuclei or other non "whole cell" preps.

Protocol: DNAse I Treatment

Materials:

- DNAse (Sigma D-4513) 100 µg/ml in Hank's Balanced Salt solution (HBSS, Sigma H-6648)
- Magnesium chloride hexahydrate (Sigma M-2670) MW=203.3
- 203 mg/ml = 1000 mM or 200X

Procedure

- Treat cells for 15 to 30 minutes in a solution of 100 µg/mL DNAse and 5 mM $MgCl_2$ in HBSS at room temperature.
- Wash the cells once in the presence of 5 mM MgCl2 in HBSS.
- Filter through Falcon® Cell Strainer (Catalog #[35]2340 or #[35]2235).
- Gently suspend the cells Stain Buffer (BSA) containing $MgCl_2$ and 25-50 µg/mL DNAse (as a maintenance dose) prior to and during the sort.

References

Crissman, HA, Mullaney, PF, and Steinkamp, JA. Methods and applications of flow systems for analysis and sorting of mammalian cells. Meth. Cell Biol. 9:175 (1975).