Minimizing Aggregates in Samples

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Why Are Single Cell Suspensions Important?

- Doublets and higher order aggregates cannot be used for analysis or sorting, because the true status of each fluorescent marker cannot be determined. The presence of aggregates means there are fewer cells available for your analysis or sort.
- Aggregates cause instability in the flow through the instrument, leading to anomalies in fluorescence intensity measurements.
- Larger or accumulated aggregates can clog the instrument, requiring interruptions to your appointment for staff to clear the clog.
- Buildup of aggregates in the instrument causes samples to run slower, making your acquisition time longer.

Addressing Common Causes of Aggregation

Identify the problem

It is important to consider the source of aggregation in your samples to properly address the issue. Adhesion can often be counteracted by removing divalent cations. However, the activity of DNAse requires divalent cations. Using EGTA instead of EDTA may allow magnesium ions to interact with DNAse while still partially mitigating adhesion.

Adhesion – often cation-dependent

- When harvesting adherent cells, quench Trypsin with Soybean Trypsin Inhibitor instead of media/serum.
- Use buffers free of divalent cations (calcium-free, magnesium-free).
- Try BSA instead of serum as a protein component. However, not all cells tolerate BSA, so test this method before starting a big experiment.
- If cells don't tolerate BSA, use dialyzed serum from which calcium and magnesium have been removed.
- Add EDTA (1-5mM) to samples. Not all cells tolerate EDTA, so test this method before starting a big experiment.

Cell Death – DNA released from lysed cells

1. Treat cells with 100ug/mL DNAse I with 5mM MgCl2 in HBSS at room temperature for 15-30 minutes.
2. Wash cells once in HBSS with 5mM MgCl2.
3. Resuspend cells in HBSS containing 1-5mM MgCl2 and 25-50ug/mL DNAse I.

References

https://www.bdbiosciences.com/documents/BD_Research_Sorting_TechBulletin.pdf

http://www.rockefeller.edu/fcrc/tips/sampleprep

http://expertcytometry.com/how-cell-culture-medium-can-decrease-cell-viability-during-a-flow-cytometry-cell-sorting-experiment/

Effects of clogs on data