DNAse to Prevent Cell Clumping

Cell clumping can result in poor sort purity when sorted target cells are attached to non-target cells, and poor recovery when coincident aborts exclude all clumped cells. DNA from lysed cells in the medium can cause cells to clump.

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 MgCl₂ in HBSS at room temperature.
- Wash the cells once in the presence of 5 mM MgCl₂ in HBSS.
- Filter the cells.
- Gently suspend the cells Stain Buffer (BSA) containing MgCl₂ and 25-50 µg/mL DNAse (as a maintenance dose) prior to and during the sort.

Notes
- DNAse I requires a concentration of at least 1 mM magnesium to work effectively, although 5 mM is optimal.
- It is important to minimize the presence of dead cells during this procedure, since actin released from dead cells irreversibly inhibits DNAse I.
- It is not recommended to combine Magnesium with EDTA

References