

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

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).