

# Technical Bulletin

## Avoiding Cell Clumping of Insect Cultures

During the adaptation of insect cells to serum-free conditions or during scale-up, insect cells tend to stick together and aggregate. This can make obtaining accurate cell counts nearly impossible and lead to inconsistent cell growth and protein expression. The following tips will help to reduce and avoid cell clumping:

- **Patience** — Cell clumping is often purely an adaptive response and will disappear over several passages.
- **Settling** — Gently swirl the cell suspension and allow the larger, heavier clumps to settle on the bottom of the container. Select the single cells from the middle of the suspension, avoiding the large aggregates.
- **Cell Density** — Dense cultures are more likely to clump. Seed cultures at  $3\text{--}5 \times 10^5$  cells/mL. Keep the total volume of media and cells at about half of the vessel's capacity. We recommend approximately 50 mL in a 125 mL flask or 100 mL in a 250 mL flask. Continue to split the cultures when they reach a density of  $2\text{--}4 \times 10^6$  cells/mL until they become completely adapted and established.
- **Oxygen** — Cells will begin to aggregate if they are deprived of adequate levels of oxygen. Always keep the caps on shaker flasks and spinner flasks slightly cracked open. Oxygen sparging will often become necessary as you scale-up to larger flasks and bioreactors.
- **Speed** — A rate of agitation that is too slow will induce clumping. Shaker cultures should use a constant speed of 120 - 140 rpm. Set the paddle speed of spinner flasks between 70 - 90 rpm for insect cultures.
- **Trituration** — Small clumps can be dissociated by gentle trituration. The cells are dissociated by flushing the cell suspension in and out of a sterile pipette. Be cautious, as harsh physical manipulation can permanently damage the cells.
- **Heparin** — As a last resort to reduce aggregation, add 10 units of heparin per mL of culture medium. Discontinue the addition of heparin once the cultures are free of clumps. The use of dextran sulfate has also been reported to reduce clumping.

For more information about this subject or other SAFC Biosciences' products and services, please call our Technical Services department.

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