



Protocol for Selenomethionine Incorporation:

This protocol assumes standard cell culture operations which are: suspension cells, grown either in shake flasks, spinner vessels or Wave units, utilizing a “complete medium” recommended for standard BEVS. Cells are assumed to be high viability, (> 98%) demonstrating robust growth rates and little to no cell clumps.

Preparation of methionine-free medium cell stock.

Transfer standard suspension cell stock, (i.e., Sf9, Sf21, or Tni cells) in mid-log growth, from standard cell culture medium to methionine-free medium. Use 15 mls of culture medium, cells should be at a density of $2-3 \times 10^6$ per ml, to inoculate 30 mls of methionine-free medium for a final 45 ml of culture. Grow cells exclusively in methionine-free medium for 2 passages, allowing cells to grow to a maximum of 4×10^6 per ml, 2×10^6 if Sf21 cells are used.

Selenomethionine Incorporation.

Split culture back to 7×10^5 in methionine-free media; wait 24 hours and infect with standard MOI. Add DL-selenomethionine at 100mgs per liter, at various time intervals, beginning no earlier than 8 hours after time of infection, then at sequential 24 hour intervals after infection up to harvest time and compare to when first additions were made at 24, 48, 72 hour intervals after infection
Harvest at day 3 or day 4 or at 50% viability.