



# Culturing of Insect Cells and Production of Baculovirus Expressed Recombinant Proteins in 2.8 Liter Fernbach System

## References:

Johansson, T., Enestam, A., Kronquist, R., Schmidt, M., Tuomiminen, N., Weiss, S.A., and Oker-Blom, C. *Synthesis of Soluble Rubella Virus Spike Protein in Two Lepidopteran Cell Lines: Large Scale Production of the E1 Protein. Journal of Biotechnology 50 (2-3): 171-180, 1996.*

The use of Fernbach flasks is an ideal method to amplify the cells and virus inoculum for the bioreactor. The method is also very practical for producing sufficient amounts of various proteins for feasibility studies using HyQ© SFX-Insect™ medium. The large surface area in this vessel enables sufficient aeration and, therefore, the cells, when they reach high density, are not oxygen depleted.

1. The seeding cell density should be  $5 - 6 \times 10^5$  cells/ml. After 5-6 days in culture, the cell concentration should reach about  $1.4 \times 10^7$  cells/ml.
2. The optimum medium volume to obtain maximum cell yield/ml is 750 ml/2.8 liter Fernbach flask. To obtain maximum budded virus yield and recombinant protein expression, use 700 – 800 ml vessels.
3. For subculturing/dispensing cell suspension from Fernbach flasks into consecutive vessels bioreactor, use a Fernbach flask with a single spigot with attached silicone tubing connecting to an aseptic filling bell. For the production of virus or recombinant proteins, a Fernbach flask without a spigot may also be used.
4. For medium exchange and cell separation/concentration using a hollow fiber cartridge, use a Fernbach flask with two spigots and a hollow fiber cartridge connected between the spigots.
5. For infection, use the cells in mid logarithmic phase, which should be approximately 3-4 days post-seeding.
6. The physical parameters for culturing the cells in Fernbach flasks are the same as for a 100 ml cell suspension in a 250 Erlenmeyer flask shaker culture.