



# Sequential Adaptation to HyQ© SFX-Insect™ Medium

## References:

Weiss, S.A., Whiford, W.G., S.F. Gorfien and G.P. Godwin. Chapter 4. *Insect Cell Culture Techniques in Serum-Containing Media*. In: *Methods in Molecular Biology, Baculovirus Expression Protocols, Vol. 39*, pp 65-78. Christopher D. Richardson, ed. Humana Press, Totowa, New Jersey, 1995.

Weiss, S.A., Godwin, G.P., S.F. Gorfien and W.G. Whiford. Chapter 4. *Insect Cell Culture Techniques in Serum-Free Media*. In: *Methods in Molecular Biology, Baculovirus Expression Protocols, Vol. 39*, pp 79-86. Christopher D. Richardson, ed. Humana Press, Totowa, New Jersey, 1995.

1. Subculture insect cells growing exponentially in a conventional medium into a 1:1 ratio of the SFX-Insect medium and serum-supplemented medium with the cell density between  $5 \times 10^5$  to  $1 \times 10^6$  viable cells/ml.
2. Incubate the cultures until viable cells undergo one to two population doublings. Subculture cells by mixing equal volume of the cell suspension in a conditioned medium and fresh SFX-Insect Medium (1:1).
3. Continue to subculture the cells in this manner until the serum concentration is reduced below 0.05%, cell viability is >85%, and the viable cell concentration is greater than  $2 \times 10^6$  cells/ml.
4. Subculture cells when viable cell concentration is increased from  $5 \times 10^5$  to  $2 \times 10^6$  cells/ml, or when  $1 \times 10^6$  to  $3 \times 10^6$  cells/ml or better is achieved.
5. For cryopreservation of the SFX-Insect adapted cells, follow the instructions described in step 6 in "Direct Adaptation to SFX-Insect Medium."
6. Recover the cells from cryopreservation, expand and check recombinant protein expression.
7. Expand recovered serum-free medium adapted cells from cryopreservation and prepare a Master Cell Bank using the lowest passage possible.