Direct Adaptation of Insect Cells to HyQ© SFX-Insect™ Medium

References:
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.

Using this method, insect cells should be adapted to HyQ SFX-Insect in 4-8 passages. However, there are some cell lines and clones that are more sensitive to the physiochemical and nutritional changes, and in some cases it may take longer than 8 passages for the adaptation. If viabilities decrease to <50%, or if the cultures are growing slowly (population doubling time is >40 hours) for more than 3-4 consecutive passages, use the sequential adaptation.

1. Prewarm HyQ SFX-Insect Medium to 25-28°C. The medium also can be left overnight at room temperature provided it is protected from light.
2. Transfer the cells grown in conventional serum-supplemented medium directly into the prewarmed SFX-Insect. The seeding density should be 5 x 10^5 to 1 x 10^6 viable cells/ml
3. When the viable cell density reaches 1 x 10^6 to 3 x 10^6 viable cells/ml, subculture the cells to 5 x 10^5 to 1 x 10^6 viable cells/ml.
4. Subculture stock cultures of SFX-Insect adapted cells 1 to 2 times per week when viable cell counts reach 2 x 10^6 to f x 10^6 viable cells/ml with at least 85% viability in about 4-7 days.
5. When the cells are adapted to SFX-Insect, viable cell counts of most insect cells should routinely exceed about 2 x 10^6 to 4 x 10^6 cells/ml. After 3-6 days in culture, the viabilities should be >85%. At this time, the adapted culture should be cryopreserved as a master seed stock. The cells should be cryopreserved in a medium consisting of 7.5% DMSO in 50% fresh SFX-Insect and 50% reconditioned SFX-Insect.
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.