



Adaptation of High Five™ Cells to HyQ© SFX-Insect™ Medium

This protocol is designed to adapt High Five™ (BTI-Tn-4) cells to serum-free suspension culture in HyQ SFX-Insect in 5 passages. The entire adaptation process takes approximately two weeks and the resulting adapted cells have doubling times of 16 and 20 hours and minimal clumping in suspension culture.

1. Rapidly thaw a 1 ml vial of High Five™ cells (Invitrogen Cat. No. B855-02) and pipette into a T-75 cell culture flask containing 15 ml of pre-warmed SFX-Insect™. Incubate the flask, cap loosened, in a humidified 27 to 29° C incubator.
2. On day 3 post-seeding, when the monolayer culture is 80 to 90% confluent, subculture the flask 1:5 by adding 3 ml of cell suspension from the original passage X + 1 culture into each of the 5 T-75s containing 12 ml of prewarmed medium. Cells are easily detached from the monolayer by tapping the flask.
3. On day 3 post-seeding, when the monolayers are about 80% confluent, detach cells from the passage X + 2 flasks, count, and setup 2 35 ml shake flask cultures at 3×10^6 viable cells/ml (in 125 ml Corning Erlenmeyer Shake Flasks) and 5 T-75s (split 1:5 as backups in case the initial adaptation to suspension culture is unsuccessful). Incubate shake flasks, caps loosened, at 27 to 29° C with a shaking speed of 150 rpm.
4. Monitor cell growth of the passage X + 3 shaker cultures and subcultures on day 3 when the suspension cultures attain a density of $2 - 3 \times 10^6$ cells/ml. If the first attempt at suspension cultures fails (minimal growth and/or viabilities <80% on day 3 to day 4), reseed another set of 35 ml shaker flask cultures at 5×10^5 viable cells/ml using the cells in the backup monolayer cultures setup in step 3, above.
5. Subculture suspension cultures every 3 days with seeding densities of 3×10^5 viable cells/ml. In three passages the cells will completely adapted to suspension culture in SFX-Insect with minimal clumping and viabilities >95%. We use total culture volumes of 75 ml in the 250 ml Corning Erlenmeyer flasks, and 150 in the 500 ml Corning Erlenmeyer flasks. We recommend maintaining the monolayer cultures as backups until the adaptations has been successfully completed. Split monolayer cultures every 3 to 4 days when they reach 80 to 90% confluency using split ratios 1:5 and 1:10.

As the cells are adapted to suspension culture, simultaneously scale up a replicate culture and cryopreserved High Five™ cells at passage X + 5 for use as your low passage master or working cell bank. Use SFX-Insect medium supplemented with 15% DMSO (final 7.5% DMSO) and 50% conditioned medium from the culture in which the cells are grown.