

1. RAPID THAW of SF9 and H5 CELLS

*Suitable for cells that were frozen at high concentration ($3-4 \times 10^7$ cells/vial) and are already adapted for suspension growth.

1. Take vial from liquid nitrogen tank.
2. Rapidly thaw in 37°C bath.
3. Spray 70% EtOH on vial.
4. Transfer the content of vial into an Erlenmeyer flask containing fresh 40ml medium. (Cells should be over 0.5×10^6 /ml)
5. Check cells the next day, and count viability.
6. Split cells to 1.2-1.5 million cells /ml
Cells are ready to work when they are doubling every 24h, and when they are uniform in size (when huge cells appear, it's a sign that the culture was in stress, and we do not recommended long term use of this culture).

2. THAWING SF9/H5 CELLS

*Suitable for cells that were frozen at $1-3 \times 10^7$ cells/vial) and are already adapted for suspension growth.

1. Take vial from liquid nitrogen tank.
2. Rapidly thaw in 37°C bath.
3. Spray 70% EtOH on vial.
4. Transfer the content of vial into 15 ml tube, containing 10ml fresh media and spin tubes in 100g/5 min
5. Aspirate medium.
6. Gently re-suspend cells in 12ml fresh medium and transfer to 125ml or 250ml Erlenmeyer flask.
7. Count cells the day after, and split to 1.2-1.5 million cells /ml
8. Count cells the next day and make sure they doubled and look viable (less than 5% dead)

3. THAWING SF9/H5 CELLS

*Suitable for cells growing in mono-layer, or for checking viability of frozen cells

1. Take vial from liquid nitrogen tank.
2. Rapidly thaw in 37°C bath.
3. Spray 70% EtOH on vial.
4. Transfer the content of vial into 25cm² flask with 10ml growing medium.
5. Incubate at 27°C incubator for 1-2 hours.
6. If cells are attached, re-feed with 6ml medium. If not wait another 2h.
7. If cells are attached incubate at 27°C/ON.
8. The next day, split 1:4 into T25cm flasks. If cells are not attached: discard cells.