Feeder Cells:

The addition of normal mouse spleen cells is recommended for establishing cultures of Hybridomas 902, 273, 500, 715, and 183 from frozen cells. Mouse spleen cell preparations do not need to be syngeneic (C57BL/10 cells are routinely used), and may be stored in culture medium (RPMI 1640 with 10% fetal bovine serum) at 4°C for up to 5 days without losing their feeder effect. Spleen cells should be prepared at a concentration of 0.5-1.0 x 10^6 cells/ml.

Thawing and Propagation of Hybridomas:

1. Swirl the vial of frozen cells gently in a 37°C water bath to thaw the cells.
2. Dilute 1.0 ml of the thawed cells into 10 ml of culture medium. Centrifuge at 1200 rpm for 5 minutes.
3. Resuspend the cells in 10 ml of culture medium (RPMI 1640 with 10% fetal bovine serum) containing 0.5-1.0 x 10^6 spleen feeder cells/ml.
4. Seed 2.0 ml of the resuspended cells into a 25 cm^2 flask and 8.0 ml of resuspended cells into a second 25 cm^2 flask.
5. Live hybridoma cells are larger and more homogeneous than dying spleen feeder cells. Grow the hybridoma cells to confluency (approximately 1 x 10^6 cells/ml) then split the cultures 1:10 or 1:20 into medium both with and without feeder cells. Do not omit the feeder cells until the culture grows well without them. Feeder cells may also be added to established cultures that have lost viability due to unintentional overgrowth, etc. Once the cultures grow well without feeder cells, they may be routinely split 1:20 twice weekly in medium without feeder cells.
6. Freeze the cells at 2-10 x 10^6 cells/ml in medium containing 40% RPMI 1640, 50% fetal bovine serum, and 10% DMSO. Thaw and seed a vial of frozen cells as described above to confirm post-thaw viability; some hybridoma lines are difficult to freeze with high viability.