SC-1/MuLV LP-BM-5

Establishing Cultures: Thaw the cells rapidly in a 37°C water bath. Transfer the thawed cells to a 25 cm^2 flask in approximately 5 ml of propagation medium. Incubate at 37° in a 5% CO_2 incubator. Split the culture when the cells become confluent. Do not allow the cells to become over-confluent.

Transferring Cells: Disperse the cells using trypsin or trypsin-versene solution. Centrifuge at 900-1000 rpm for 10 minutes, then resuspend the cells in fresh propagation medium. Seed fresh cultures at 1:10-1:100, depending upon when cultures are to be used.

Virus Pools: Create pools of LP-BM5 by mixing $1x10^6$ infected cells from an established culture with $1x10^6$ uninfected SC-1 cells in 40 ml of fresh propagation medium in a 150 cm^2 flask. Change the medium on days 1, 3, and 5, replacing with 30 ml of fresh medium. Harvest virus on day 6.

Virus Stocks: Virus pools may be prepared from supernatant fluid harvested on the day after a medium change, when the cultures are fully sheeted but not over-confluent. The contributor routinely harvests infected cells as well by scraping the cells into a small volume of supernatant, freezing and thawing, then homogenizing the cells by 2-3 careful aspirations with a syringe and 22 gauge needle (avoid creating bubbles or foam). The homogenized cells are then re-suspended in the remaining supernatant fluid and clarified by centrifugation at 2500 rpm for 20 minutes. The viral preparation must be kept cold during the entire procedure.

Freezing Cells: Infected cells should be frozen in liquid nitrogen and reconstituted as needed because yields of infectious virus may decrease on prolonged passage.