

# Protocol for Passaging Suspension Cells

Subculture cells when they are in log-phase growth. The recommended density of different cells before passaging is also different.

- 1) When the cells are in log-phase growth and ready for passaging, take a sample for counting the number of cells. If cells have settled down before taking the sample, swirl the flask to distribute the cells evenly.
- 2) Determine the total number of cells and percent viability.
- 3) Calculate the volume of medium that needed for diluting the cells to seeding density to add to dilute the culture down to the recommended seeding density.
- 4) Add the appropriate volume of pre-warmed growth medium into the culture flasks, then place the cells to the incubator.
- 5) Loosen the caps of the culture flasks to allow for proper gas exchange unless vented flasks with gas-permeable caps are used and return the flasks to the incubator.

**[Note]: To minimize the accumulation of cell debris and metabolic waste by-products in cultures, gently centrifuge the cell suspension at  $500 \times g$  for 5 to 10 minutes, and resuspend the cell pellet in fresh growth medium once every three weeks (or as needed).**