Cell culture protocol 10: Cell passage

The following protocols describe general procedures for subculturing mammalian cells in suspension culture.

For passaging your own cell line, we recommend that you closely follow the instructions provided with each product you are using in your experiments.

Passaging Suspension Cultures
Subculturing suspension cells is somewhat less complicated than passaging adherent cells. Because the cells are already suspended in growth medium, there is no need to treat them enzymatically to detach them from the surface of the culture vessel, and the whole process is faster and less traumatic for the cells. Replacement of growth medium is not carried out in suspension cultures; instead, the cells are maintained by feeding them every 2 to 3 days until they reach confluency. This can be done by directly diluting the cells in the culture flask and continue expanding them, or by withdrawing a portion of the cells from the culture flask and diluting the remaining cells down to a seeding density appropriate for the cell line. Usually, the lag period following the passaging is shorter than that observed with adherent cultures.

Suspension Culture Vessels
Suspension cultures can be maintained in sterile culture flasks (e.g., shaker flasks without baffles) that are not tissue-culture treated; however, spinner flasks (i.e., stirrer bottles) specifically designed for suspension cell culture allow for superior gas exchange and permit higher volumes of cells to be cultured.

Spinner flasks have two basic designs; the medium is agitated (i.e., stirred) by a hanging stir-bar assembly or with a vertical impeller. The vertical impeller provides better aeration. The total culture volume in a spinner flask should not exceed half of the indicated volume of the spinner for proper aeration (e.g., a 500 mL spinner should never contain more than 250 mL of culture).

Experiment materials:
- Culture vessels containing your suspension cells
- Shaker flasks without baffles or spinner bottles (see Suspension Culture Vessels)
- Complete growth medium, pre-warmed to 37°C
- 37°C incubator with humidified atmosphere of 5% CO2
- Magnetic stir plate (if using spinner flasks), roller rack (if using roller bottles), or shaking platform (if using conventional culture flasks or petri dishes)
- Reagents and equipment to determine viable and total cell counts (e.g., Countess Automated Cell Counter, Trypan Blue and hemacytometer, or Coulter Counter)
Protocol for Passaging Suspension Cells

All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood. Subculture cells when they are in log-phase growth before they reach confluency.

The maximum recommended cell density before passaging varies with cell lines; refer to the cell-specific product insert or manual for details.

Cells Grown in Shaker Flasks

The following protocol describes a general procedure for passaging mammalian cells grown in suspension culture using shaker flasks in a shaking incubator. For detailed protocols, always refer to the cell-specific product insert.

Note: Make sure that the shaker flask does not have baffles (i.e., the indents at the bottom of the flask designed to provide agitation), because they ruin the shaking rhythm.

1. When the cells are ready for passaging (i.e., log-phase growth before they reach confluency), remove the flask from the incubator and take a small sample from the culture flask using a sterile pipette. If cells have settled down before taking the sample, swirl the flask to evenly distribute the cells in the medium.

2. From the sample, determine the total number of cells and percent viability using the Countess Automated Cell Counter or a hemacytometer, cell counter, and Trypan Blue exclusion.

3. Calculate the volume of media that you need to add to dilute the culture down to the recommended seeding density.

4. Aseptically add the appropriate volume of pre-warmed growth medium into the culture flask. You may split the culture to multiple flasks if needed.

5. Loosen the caps of the culture flasks one full turn to allow for proper gas exchange (or use a gas-permeable cap), and return the flasks to the shaking incubator. The shaking speed depends on the cell line.

Note: To minimize the accumulation of cell debris and metabolic waste by-products in shaker cultures, gently centrifuge the cell suspension at $100 \times g$ for 5 to 10 minutes, and resuspend the cell pellet in fresh growth medium once every three weeks (or as needed).

Cells Grown in Spinner Flasks

The following protocol describes a general procedure for passaging mammalian cells in suspension grown using spinner flasks. For detailed protocols, always refer to the cell specific product insert.

Note that cells are sensitive to physical shearing. Ensure that impeller mechanisms rotate freely and do not contact vessel walls or the base. The top of the paddles should be slightly above the medium to ensure adequate aeration to the culture. Adjust the spinner mechanism so that paddles clear the sides and the bottom of the vessel. The table below lists the minimum volumes of media needed for different spinner flask sizes.

We do not recommend initiating a spinner culture into a spinner flask larger than 500 mL. We suggest scaling up from smaller spinners that have already been established.

1. When the cells are ready for passaging (i.e., log-phase growth before they reach confluency), remove the flask from the incubator, and take a small sample from the culture flask using a sterile pipette. If cells have settled down before taking the sample, swirl the flask to evenly distribute the cells in the medium.

2. From the sample, determine the total number of cells and percent viability using the Countess Automated Cell Counter or a hemacytometer, cell counter, and Trypan Blue exclusion.

3. Calculate the volume of media that you need to add to dilute the culture down to the recommended seeding density.

4. Aseptically add the appropriate volume of pre-warmed growth medium into the culture flask. You may split the culture to multiple flasks if needed.

5. Loosen the side arm caps of the spinner flasks...
one full turn to allow for proper gas exchange, and return the flasks to the incubator. The spinner speed depends on the cell line and the impeller type. Make sure that the spinner speed is kept within the recommended values to avoid damage to the cells from shear stress.

**Note:** To minimize the accumulation of cell debris and metabolic waste by-products in spinner cultures, gently centrifuge the cell suspension at $100 \times g$ for 5 to 10 minutes, and resuspend the cell pellet in fresh growth medium once every three weeks (or as needed).

Notes on Subculturing Suspension Insect Cells

While the general procedure for subculturing insect cells follows the same steps as mammalian cells, some key requirements of these culture systems are different. For best results, always follow the instructions provided with the insect cell lines you are using in your experiments.

- It is not necessary to change medium when you are culturing cells in suspension. Regular subculturing requires the removal of cell suspension and the addition of medium sufficient to dilute culture to the appropriate density (refer to the cell specific product insert). Adding fresh medium is sufficient to replenish cell nutrients.
- CO2 exchange is not recommended for insect cell culture.
- Maintain insect cells at 27°C in a non-humidified environment. Cells can be maintained at room temperature on the bench top or in a drawer, however, a 27°C controlled environment is recommended.
- Use media specifically formulated for insect cell growth.
- Use a surfactant to decrease shearing. 0.1% Pluronic F-68 is recommended for spinner insect cultures. Pluronic F-68 (BASF) is a surfactant that decreases cell membrane shearing due to impeller forces.

**Note:** Sf-900 II SFM and Express Five SFM already contain surfactants.

Certain insect cell lines may require adaptation to suspension culture. For more information, refer to the cell-line specific product insert or manual.

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