Sep 24, 2025

Sub-culturing of cells

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6z3okgqe/v1

Mohit Sharma[1]

[1]Jiwaji University

M Mohit Sharma

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6z3okgqe/v1

Protocol Citation: Mohit Sharma 2025. Sub-culturing of cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6z3okgqe/v1

Protocol status: Working
We use this protocol and it's working

Created: September 24, 2025

Last Modified: September 24, 2025

Protocol Integer ID: 228072

Keywords: Cell Culture, Subculturing of cells, Animal cell culture, cells trypsinization, culturing of cells trypsinization, process of cell dissociation, trypsinization process, cell culture, cell dissociation, proteolytic enzyme, adherent cells from the vessel, trypsinization, adherent cell, cell, protein, complete the cell, using trypsin, trypsin, process

Abstract

Trypsinization is the process of cell dissociation using trypsin, a proteolytic enzyme which breaks down proteins, to dissociate adherent cells from the vessel in which they are being cultured. When added to a cell culture, trypsin breaks down the proteins that enable the cells to adhere to the vessel. When the trypsinization process is complete the cells will be in suspension and appear rounded.Trypsinization is the process of cell dissociation using trypsin, a proteolytic enzyme which breaks down proteins, to dissociate adherent cells from the vessel in which they are being cultured. When added to a cell culture, trypsin breaks down the proteins that enable the cells to adhere to the vessel. When the trypsinization process is complete the cells will be in suspension and appear rounded.

Materials

- Culture vessels containing adherent cells
- Tissue-culture flasks, plates or dishes
- Complete growth medium, pre-warmed to 37°C
- Disposable, sterile 15-mL tubes
- 37°C incubator with humidified atmosphere of 5% CO 2
- Balanced salt solution such as Phosphate Buffered Saline (PBS), containing no calcium, magnesium, or phenol red
- Dissociation reagent such as trypsin
- Equipment to determine viable and total cell counts such as hemacytometer or Coulter Counter

Before start

Sterile conditions must be maintained to prevent any contamination to the cells.

1 Select a 25cm^2 culture flask, containing a confluent monolayer of cells.

2 Remove and discard the spent cell culture media from the culture vessel.

3 Wash cells using Phosphate Buffered Saline (approximately 2 mL per 25 cm ^{2} culture surface area). Gently add wash solution and rock the vessel back and forth several times.

4 Remove and discard the wash solution from the culture vessel.

5 Add the dissociation reagent such as trypsin (approximately 1 mL per 25 cm ^{2} culture surface area) to the side of the flask. Gently rock the container to get complete coverage of the cell layer.

6 Incubate the culture vessel at room temperature for approximately 2 minutes.

7 Observe the cells under the microscope for detachment. You may also tap the vessel to expedite cell detachment.

8 When ≥ 90% of the cells have detached, tilt the vessel for a minimal length of time to allow the cells to drain.

9 Add the pre-warmed complete growth medium to dilute cell suspension and dispense into new cell culture vessels

10 The split ratio 1:4 depending upon the concentration of the cells.

11 Incubate the cells at 37 °C till a confluent monolayer is complete.