



SUBCULTURING PROTOCOL

Prepare to subculture the cells when the culture has reached around 80% confluence. The following protocol is for adherent cells; for suspension cells, start at step 6.

Protocol

1. Pre-warm the complete growth media specified by the cell line datasheet in a 37°C water bath. Pre-warm the Trypsin-EDTA ([TM050](#)) to room temperature.
2. Carefully aspirate the culture media from the culture vessel without disturbing the cell monolayer.
3. Add pre-warmed Trypsin-EDTA to the culture vessel. Gently rock the culture vessel to ensure complete coverage of the Trypsin-EDTA over the cells. (Table 1 provides guidelines to the volumes of Trypsin-EDTA needed for a range of culture vessels).
4. Observe the cells under a microscope to confirm they are dissociating from each other and are rounding up. Gently tap the culture vessel from several sides to promote cell detachment. Cells that are difficult to detach can be put in 37°C for several minutes to facilitate dispersal.
5. When majority of the cells have detached, add an equal volume of the complete media into the culture vessel to neutralize the trypsin-EDTA. Gently swirl or pipette the culture suspension to ensure the neutralization is complete.
6. Transfer the culture suspension to a sterile centrifuge tube.
7. Centrifuge the cell suspension at 1500 rpm for 3 minutes. The actual centrifuge duration and speed may vary depending on the cell type.
8. Aspirate the supernatant after checking all cells are pulled down into the pellet. Re-suspend the cell pellet in pre-warmed fresh complete media (adjust volume as necessary according to Table 1).
9. Seed new culture vessels at an appropriate cell density.
10. Place the newly seeded culture vessel in a 37°C, 5% CO₂ incubator. Incubate for at least 24 – 48 hours before processing the cells for downstream experiments.
11. Renew the culture media every 2-3 days if the cells have not reached 80% confluency.

Table 1: Volume of Trypsin-EDTA and Culture Media for Different Culture Vessels

Culture Wares	Trypsin-EDTA Volume	Complete Media Volume
12- well plate	0.5 ml/ well	2.0 ml/ well
6- well plate	1.0 ml/ well	3.0 ml/ well
T-25 flask	3.0 ml/ flask	7.0 ml/ flask
T-75 flask	5.0 ml/ flask	20.0 ml/ flask
100 mm dish	3.0 ml/ dish	10.0 ml/ dish
150 mm dish	5.0 ml/ dish	20.0 ml/ dish

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