3t3 Cell Culture Protocol (ATCC CCL-98)

Characteristics –
Organism – mouse
Tissue – embryo
Cell type – fibroblast
Growth Properties – adherent
Biosafety Level - 1

Materials

- Performance FBS (Life Technologies)
- DMEM (ATCC, 30-2002)
- Pen/Strep (Life Technologies)
- Trypsin-EDTA (Life Technologies)

Media Formulation

- Complete Growth Media – 1% PenStrep (v/v, 5 mL) + 10% (v/v, 50 mL) FBS + 89% (v/v, 445 mL) DMEM
- Cryoprotectant Medium – Complete growth media + 5% (v/v) DMSO ($1*10^6$ cells / mL)

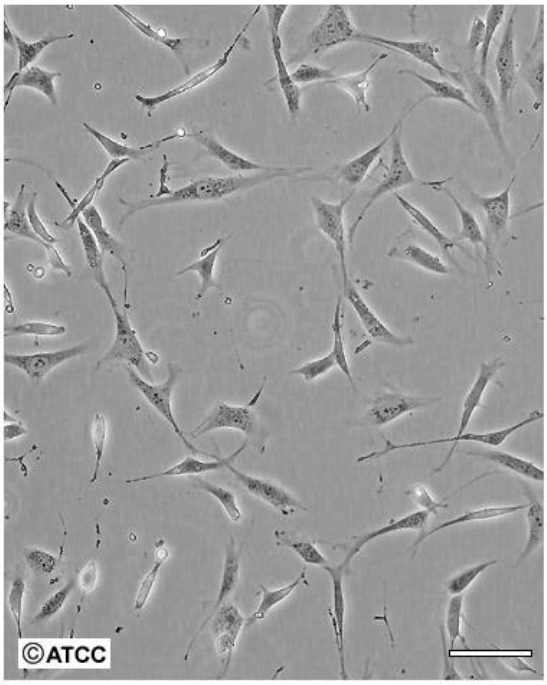
Initiating Culture

1. Place a T-75 with complete growth medium into an incubator for at least 15 mins to allow the medium to reach its normal pH.
2. Thaw the vial by gentle agitation in a 37 C water bath (should take less than two mins)
    a. note – keep the O-ring and cap out of water
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium, and spin at 600g for 5 mins
4. Resuspend cell pellet with the complete and dispense into the culture flasks from step 1.
5. Incubate the culture

Subculture

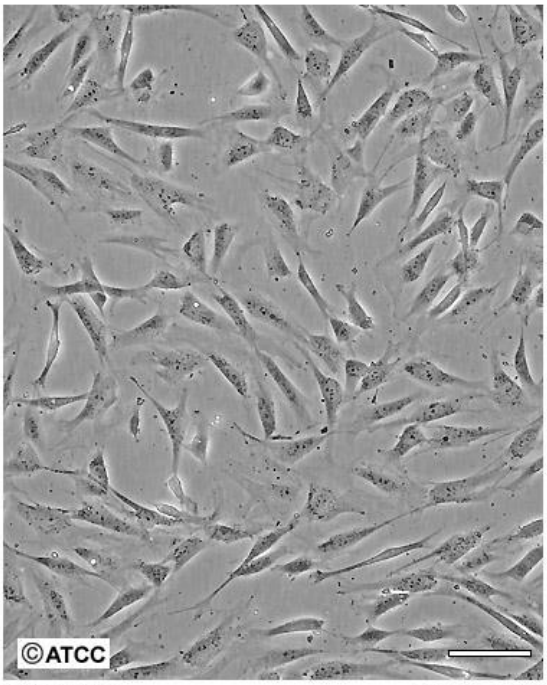
1. Remove and discard culture medium
2. Rinse the cells with PBS
3. Add 3mL (T-75) or 6mL (T-150) of Trypsin-EDTA solution and incubate at 37 C and observe after 5mins. Incubate for longer period of time until all the cells are lifted.
    a. Do not agitate the cells by hitting or shaking the flask while waiting for cells to detach.
4. Add 6-8 mL of complete growth medium
5. Spin the cells at 600g for 5 mins and aspirate by gently pipetting
6. Resuspend cells in appropriate volumes of media
7. Add appropriate aliquots of the cell suspension to new culture flasks
    a. A subcultivation ratio of 1:3 is recommended or 5×10^3 cells/cm^2
8. Incubate cultures at 37 C
9. Change the media twice a week.

ATCC Number: **CRL-1658**
Designation: **NIH/3T3**



Low Density

Scale Bar = 100µm



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