

NIH-3T3 Tissue Culture Methods

Note: obtained cells from ATCC (cat# is CRL-1658)

Need:

- DPBS (- Calcium, - Magnesium)
- TrypLE Express (cat# 12604021, Gibco 500 ml)
- Corning Hyperflask (cat # 10030)

T75 cm² Flasks (Splitting cells):

1. Take an 80% confluent T75 cm² flask from the 37°C/5% CO₂ incubator and remove the old media.
2. Add 10 ml of DPBS to the flask, wash the cell area, and then remove.
3. Add 5 ml of TrypLE Express to the flask and place it back into the incubator for 3-5 minutes (until cells detach from flask).
4. Remove the flask from the incubator. Then add 10 ml of 3T3 flask media to the flask.
5. Take cell suspension from flask and place in 50 ml conical tube, centrifuge at 1,000 rpm for 5 minutes at 24°C.
6. Carefully remove the supernatant and resuspend pellet in 5-6 ml of 3T3 growth media.
7. Add 1 ml cell suspension to T175 cm² flask containing 40 ml 3T3 growth media
8. Feed every other day with 40 ml fresh 3T3 growth media.

Making conditioned Media:

1. Take an 80% confluent T175 cm² flask from the 37°C/5% CO₂ incubator and remove the old media.
2. Add 20 ml of DPBS to the flask, wash the cell area, and then remove.
3. Add 15 ml of TrypLE Express to the flask and place it back into the incubator for 3-5 minutes (until cells detach from flask).
4. Remove the flask from the incubator. Then add 10 ml of 3T3 flask media to the flask.
5. Take cell suspension from flask and place in 50 ml conical tube, centrifuge at 1,000 rpm for 5 minutes at 24°C.
6. Carefully remove the supernatant and resuspend pellet in 10 ml of 3T3 growth media/flask
7. Add 10 ml of cell suspension to 500 ml of 3T3 growth media, and fill Hyperflask,
8. When cells reach confluence remove old media and add fresh 3T3 Growth media to fill Hyperflask.
9. Allow media to remain in flasks for 10 days. Then collect the media in a sterile bottle.
10. Filter media using a Vacucap filter or Corning Filter system (this will take awhile and may require more than one filter due to cells clogging old filter).
11. Conditioned media is good for one month or can be aliquoted and frozen for future use.

Coating flasks and Filters with Conditioned media:

- For T25 cm² Flasks add 2 ml conditioned media for at least 4 hours in 37°C CO₂ incubator.
- For T75 cm² Flasks add 5 ml conditioned media for at least 4 hours in 37°C CO₂ incubator.
- For T225 cm² Flasks add 10 ml conditioned media for at least 4 hours in 37°C CO₂ incubator.
- For Transwell Filters (cat# 3470) add 100 µl to top of filter for at least 4 hours in 37°C CO₂ incubator.
- For HTS Transwell 24 Well plates (cat# 3378) add 100 µl conditioned media to top of filter for at least 4 hours in 37°C CO₂ incubator.
- For Snapwell Filters (cat# 3801) add 200 µl conditioned media to top of filter and 1 ml for at least 4 hours in 37°C CO₂ incubator.
- After 12 hours, remove conditioned media and either use culture ware immediately or store at 4°C (must be used in one week, though usually I prefer to only make enough culture ware for immediate use).

NIH-3T3 Media Compositions + Protocol (Per Vertex Protocol)

Need:

- DMEM (cat# 11965-092, Invitrogen 500 ml)
- Fetal Bovine Serum (FBS) [characterized] (cat # SH30071.03, Hyclone 500 ml)
- Sodium Pyruvate [100 mM,100X] (cat # 11360-070, Gibco 100 mM)
- MEM Non-essential Amino Acids Solution [10 mM,100X] (cat # 1140-050, Invitrogen 100 ml)
- HEPES Buffer Solution [1 M] (cat# 15630-080, Invitrogen 100 ml)
- Pen-Strep [10,000 units Penicillin/10,000 units Streptomycin] (cat # 15140-122 Invitrogen 100 ml)

Growth Media [Flask Media]

1. Take a 500 ml bottle of DMEM and remove 50 ml (discard).
2. Add the following to the DMEM:
 - 50 ml of Fetal bovine serum (final concentration is 10%).
 - 5 ml of 100X MEM Non-essential Amino Acids Solution (a 1:100 dilution, final concentration is 1X).
 - 5 ml of 100X Sodium Pyruvate (a 1:100 dilution, final concentration is 1X).
 - 5 ml of 1 M HEPES Buffer Solution (a 1:100 dilution, final concentration is 10 mM).
 - 5 ml of Pen-strep (a 1:100 dilution, final concentration is 100 units Penicillin/100 units Streptomycin).
3. Filter media with Corning Filter system or VacuCap Filter