

Thomson Lab Protocols

Embryonic Bodies

1. Let human ES cells grow until the colonies are large and the cells are pretty piled up - about the time when you would normally split or even a day past that.
2. Treat cells with 0.2 - 0.5 mg/ml Dispase. You want to use the lowest possible concentration of Dispase, but it tends to vary a bit.
3. Wait until the colonies completely detach from the plate. Do not blow colonies off with a pipet. This should take about 20-30 min. If nothing is happening by that point, add more Dispase.
4. Once the colonies come up, gently transfer them to a 15ml conical tube with a 10ml pipet. You don't want to break up the colonies.
5. The cells should sink to the bottom of the tube after a minute or two without any spinning. Aspirate off the media and wash once in hES media. If you are in a hurry and need to spin the colonies down, 1 min at 500 rpm is enough.
6. Transfer cells to a flask containing ES media without bFGF. Put all of the EBs from one 6 well plate into a T80 flask with about 25mls media.
7. The cells will round up into actual embryoid bodies after about 12-24 hrs. They should then be fed every day by exchanging half the media with fresh media. The EBs should not attach, if they do, gently tap the flask to dislodge the EBs.

Notes:

- Dispase is dissolved in DMEM-F12 basal media.

General notes on ES cell culture

- hES media has a two week shelf life.
- hES cells should be cultured in 4, 6, 24, 48, 96 well plates. Growing cells in flasks is not recommended because it is very difficult to scrape cells in flasks.
- hES cells need to be fed every day with hES media. You can add 2 volumes of media and then not feed for 48 hours (ie. over the weekend.)
- You should pick differentiated cells off of the plate if more than 5% of your culture is differentiated.
- Alternatively, you can pick to keep the good colonies if there is massive differentiation within your culture.