Thomson Lab Protocols

Splitting Human ES Cells in Defined Media

The Thomson lab currently uses TeSR media, but this protocol will work with other defined medium

1. Warm 2mg/ml Dispase to 37 °C.
2. Aspirate media off of cell culture plate.
3. Add the following amount of Dispase (2mg/ml in DMEM/F12):
   - 0.5ml/well of 4 well plate
   - 1.0ml /well of 6 well plate
4. Incubate at 37 °C with 5% CO2 for 5-7 minutes.
5. During 5 minute incubation, replace media on Matrigel coated plate (see Matrigel coating procedure) with fresh, ES cell culture media.
6. After 5 minutes of incubation, check cells to determine if they are ready to be passaged. (Cells are ready for passaging when the majority of colonies are beginning to round up away from the plate. If cells begin to float off the plate, you will need to collect the cells and Dispase; spin them down by centrifuging at 1000 rpm for 5 minutes, washing (with repeat centrifuging) twice with DMEM/F12, and proceed to step 9)
7. If cells are not ready to be passaged, return them to incubator for 1-3 minutes.
8. Wash cells (on plate) 3 times with sterile DMEM/F12. Gently add media as Dispase treated cells will easily wash off plate.
9. Resuspend cells in appropriate volume of media for plating:
   - 3.5 ml for 6 well plate
   - 1 ml for a 10cm dish
10. Gently break up cells by pipeting up and down 1-2 times.
11. Plate cells onto new plates (for a 6 well plate, add 0.5ml/well and distribute the final 0.5ml dropwise to each well for even cell distribution.
12. Place cells in incubator.

Notes:

- Dispase splitting solution lasts approximately 2 weeks and the fresher the solution, the less time it takes for colonies to up off the plates.
- There is an “art” of cell culture splitting. Cells should be Dispased to effect. Cells shouldn’t need to be harshly scraped off of the plates (as is acceptable in feeder layer culture conditions). Cells should be enzymatically treated until the colonies easily slough off the plate. This will give the user the ability to control the colony size. Too little treatment and the resulting harsh scraping will kill many cells.
- Do not skip wash steps! Any amount of residual Dispase will inhibit cell attachment.
- Cells should be passaged as larger colonies using this media system than using MEFs. Therefore, do not break up colonies as much by pipeting up and down as many times.