Embryoid Body Formation

Background

Embryoid Body (EB) culture is used to examine the differentiation potential of the embryonic stem (ES) cell line. The cells are grown using low-attachment dishes in the presence of complete growth medium lacking leukemia inhibitory factor (LIF). This process induces differentiation, permits suspension culture, and causes the cells to form aggregates. These aggregates are allowed to grow for several days or weeks and samples may be taken at various time points for analysis by flow cytometry or antibody staining. The following protocol was established for the formation of EBs from mouse embryonic stem cells and should not be used for human embryonic stem cells.

Protocol

1. In the days prior to EB formation, grow up 2 to 3 confluent T-75 flasks of the ES cells containing the minimum amount of fibroblasts.
2. Hydrate 10 x 10-cm ultra-low-attachment dishes (Corning #3262) according to manufacture's instructions.
3. Remove the medium from the T-75 flasks and wash with 10 ml of 1X PBS without Ca or Mg (ATCC® SCRR-2201).
4. Add 5 ml of 0.25% Trypsin/0.53 mM EDTA (ATCC® 30-2101).
5. Incubate at 37 °C for ~2 minutes or until cells detach. Lightly tap the sides of each flask to help detach cells from the surface.
6. Inactivate the trypsin with an equal volume of medium containing serum.
7. Pipette the cell suspension in order to form a single cell suspension.
8. Spin down the cells at 270 x g for 5 minutes.
9. Remove the supernatant and resuspend the cells in ES medium.
10. Add the cell suspension back into the old flasks and incubate for 15-30 minutes. (The majority of residual MEFs should reattach.)
11. Collect the cells from the flasks into one or more 50-ml tubes.
12. Rinse each flask lightly with 5 ml of ES medium without LIF and add the additional volume to the tubes. Try to leave the MEFs behind.
13. Perform a cell count to determine the number of ES cells.
14. Seed the cells (2 x 10^6/dish) onto fibroblast-free, 10-cm Corning Ultra-Low-Attachment Dishes (Corning Catalog No. 3262) containing 10 ml of ES medium without LIF.
15. Add additional ES medium without LIF to each dish to bring the final volume to 15 ml.
16. After 24 hours, change the medium.
    a. Spin EBs down at 270 x g for 5 minutes. Keep the dishes for later.
    b. Remove the supernatant and resuspend in 10 ml of ES medium without LIF.
    c. Add 15 ml of ES medium without LIF to each of the 10 dishes.
    d. Evenly distribute the EBs among the dishes (1 ml per dish).
17. Continue to culture the EBs and change the medium every other day. Except for the first medium change, change the medium by tilting the dish so that the EBs settle and remove as much medium as possible without removing the EBs. Replace the volume removed with fresh medium without LIF.
18. EBs will spontaneously differentiate and can be collected at various time points for analysis by flow cytometry or immunostaining.
19. For long-term EB culture, it is recommended that you transfer the EBs to new dishes at day 14.

Preparation for Flow Cytometry

1. Dissociate EBs to single-cell suspension using 0.2% collagenase (Roche 103578) or 0.05% trypsin (ATCC® 30-2101).
2. Collect and pool 2 or 3 dishes of EBs into a 50-ml tube.
3. Pellet EBs by centrifugation or letting them settle.
4. Remove the medium and add approximately 5 ml of collagenase or trypsin per 10-cm dish of EBs.
5. Incubate the tubes at 37ºC for 5 minutes.
6. Pipet the suspension up and down. Observe a small sample under the microscope to check for a single cell suspension.
7. If the EBs have not dissociated to single cell, return the tube to 37ºC. Check periodically for dissociation to single cell. The amount of time required is related to the size of the EBs and the enzyme solution chosen. The later the time point, the longer the incubation required. Also, trypsin does not require as long an incubation time as Collagenase, but be careful not to lyse the cells.
8. Once most of the EBs are dissociated, dilute the enzyme 1:1 with serum-containing medium and follow the protocol “Flow Cytometry for Embryonic Stem Cells”.