GRADIENTECH

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Protocol for neuralepithelial-like stem cells (lt-NES) migration studies in CellDirector® 2D

Make sure all coating solutions, or the PBS that the coating is diluted in, is equilibrated in a cell incubator 37 °C over night to avoid bubble formation. The PBS used for washing should also be equilibrated.

Protocol for differentiating iPS cells into lt-NES cells: Falk, A *et al.* (2012) PLoS ONE 7(1): e29597

Coating of CellDirector 2D (a two-step procedure)

1. Add 200 µl of a Poly-L-Ornithine coating solution (100 µg/ml in sterile PBS, product number P2533 or P3655, Sigma) to the empty CellDirector 2D assay.
2. Place the CellDirector 2D assay with the green side facing up in a 10 cm Ø Petri dish and incubate 30 min in the cell incubator at 37 °C.
3. Wash one time with 200 µl sterile PBS: pipett slowly to only remove the access amount of the poly-l-ornithine coating layer.
4. Add 200 µl of a laminin coating solution (10 µg/ml in sterile PBS, product number: L2020, Sigma). Pipett slowly.
5. Incubate 2-3h in the cell incubator 37 °C.
6. Wash one time with 200 µl sterile PBS: pipett slowly to only remove the access amount of the laminin coating layer.

Cell Loading

1. Start by diluting the lt-NES cells to a concentration of 1 x 10^6 cells/ ml.
2. Add 200 µl of the cell suspension to the coated CellDirector 2D assay by slowly pipetting. Let the cells adhere in the cell incubator for 2-4 h. Check that the cells have adhered by looking in a microscope, the CellDirector can remain within the Petri dish when using the inverted microscope.
3. Fill syringe 1 with cell media (DMEM:F12/N2 medium, see Falk et al 2012). Fill syringe 2 with the cell media containing your chemoattractant.
4. Start the CellDirector 2D experiment as described in the manual.
5. Take a note of the orientation of the CellDirector assay and on which side the chemoattractant is put on.
6. Collect images using bright-field microscopy (10x objective) at 0.2 fpm (1 image every 5 min).

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