Protocol for PC3 cell migration in a 0-10% serum gradient in CellDirector® 2D

Make sure that all solutions including the cell media, the coating solutions and the PBS that the coating is diluted in, are equilibrated overnight in a 37 °C cell incubator, to avoid any bubble formation. Remember to put the equilibrated solutions back in the incubator shortly after use.

Preparation of cells

1. Culture PC3 cells in BioWhittaker™ Ham's F-12 medium (Lonza) supplemented with 10% FBS and 2mM L-glutamine at 37°C in a humid atmosphere with 5% CO_2.
2. Starve the PC3 cells in medium with 0.5% FBS over night.
3. Trypsinize the cells and deactivate the trypsin with medium with 0.5% FBS.
4. Centrifuge the cells.
5. Count the cells and take the desired number of cells to a new tube.
6. Centrifuge again and re-suspend in medium with 0.5% FBS (equilibrated medium) to $1x10^6$ cells/ml.
7. Tap gently on the pellet to reduce the up-down pipetting required for mixing. Avoid introduction of bubbles at this step.

CellDirector2D coating and cell loading

1. Add 200 µl of type I collagen (200 µg/ml in equilibrated PBS) to an empty CellDirector 2D.
2. Put the CellDirector assay, with the green side facing up in a 10 cm Ø Petri dish and incubate 1h in the cell incubator at 37 °C.
3. Add 200µl of your cell suspension (slowly pipetting) containing 200U/ml penicillin-streptomycin (optional). Let the cells adhere in the cell incubator for 1h. Check that the cells have adhered by looking in a microscope. The CellDirector assay can remain within the Petri dish when using the inverted microscope.
4. Prepare equilibrated medium with 0% FBS (control) and medium with 10% FBS (chemoattractant).
5. Start the CellDirector 2D experiment as described in the manual.
6. Take a note of the orientation of the CellDirector assay and on which side the chemoattractant is put on.
7. Collect images using bright-field microscopy (10x objective) at 0.2 fpm (1 image every 5 min).

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