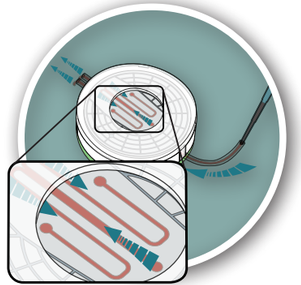

**1. Equilibrate media**

» Place all cell media overnight in an incubator in a slightly open vial (for temperature equilibration and degassing).

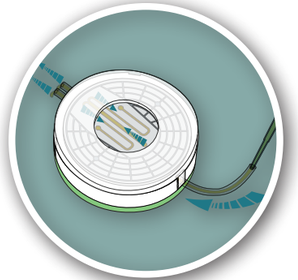


**2. Apply coating**

» CellDirector 2D is delivered uncoated (glass). Depending on the adherent cells, the assay needs to be coated prior to cell seeding.

» Slowly pipet 200 µl of your coating solution into the outlet tube.

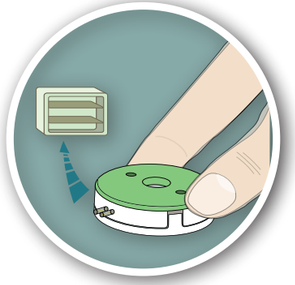
» Incubate at room temperature (10-60 min).



**3. Cell seeding**

» Prepare your cell suspension as usual (0.5-1x$10^6$ cells/ml recommended for chemotaxis experiments).

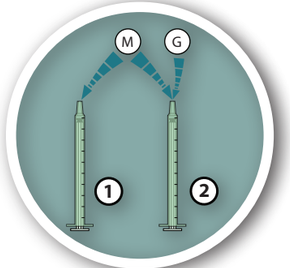
» Slowly pipet 200 µl cell suspension into the outlet tube.



**4. Adhesion of cells**

» Place CellDirector 2D in a sterile petri dish and place in humidified incubator.

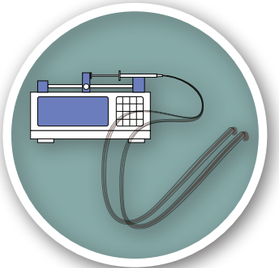
» Incubate until the cells have adhered (60min, confirm with microscope).




**5. Syringes**

» Fill syringe (1) with cell media (M), and syringe (2) with cell media and gradient substance (G).

» Connect the needles to the syringes and fill up the tubes.



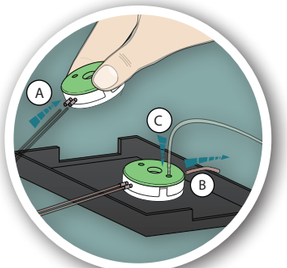
**6. Syringe pump**

» Fit the syringes into the syringe pump (e.g. Fusion 100, REF 90-001).

» Select the BD 1ml Plastic syringe in the syringe library (ID 4.78 mm).

» Start pump – ensure the tubes contain no air bubbles and media is exiting tube ends.

» Set flow rate to 1 µl/min.




**7. Start the experiment**

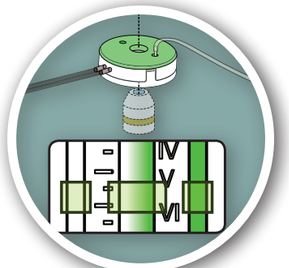
» Tilt CellDirector 2D until inlet connectors are completely filled (A).

» Insert both tubes until fully bottomed and resistance is noticable.

» Place CellDirector 2D under the microscope (CellDirector Holder, REF 70-001).

» Remove the outlet tube by pulling it gently (B).

» Connect the vacuum pump (C).




**8. Image aquisition**

» Set the 3 positions from where to collect images (neg control, gradient, pos control).

» Collect images every 30 s (fast responding cells) up to every 10 min (slow responding cells).

» Continue to collect images during the entire experiment (30 min up to 16 h).