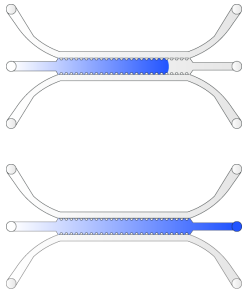
DAX-1 Quick Start Guide

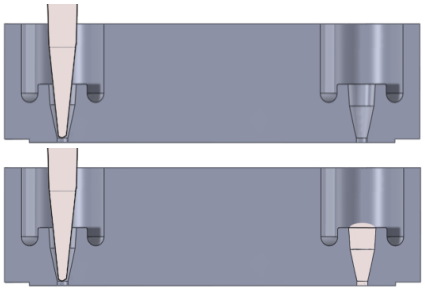
1 Preparing & Filling Gel



- Mix 10X PBS, collagen I, 0.5 M NaOH solution and deionized water in a microcentrifuge tube thoroughly on ice, according to the pre-determined collagen gel recipe. You may use any hydrogel relevant to your specific application.
- Fill **10 µl** of collagen solution from either one of the gel inlets and stop near the end of posts.
- Fill from the other gel inlet until the gel fronts merge.
- Place the gel-filled chips (on AIM holder or in humidified chambers) into a 37 °C incubator and incubate for 30 min to allow the gel to polymerize completely.

  ! Critical *Chips with unpolymerized gel must be handled with care. Excessive agitation or impact may cause unpolymerized gel to leak out of the gel channel.*

2 Hydrating & Coating Media Channels



- After incubation, insert a pipette tip into either inlet of the media channel that requires coating and push gently until the tip fits. Inject 15 µl of coating solution (e.g. 50 µg/ml fibronectin solution diluted in culture medium or 1X PBS) into the channel. Repeat this step for the opposite channel. Use culture medium instead if coating is not required.
- Incubate for 1 h or optimized time for other coating solutions in a 37 °C incubator.
- Add 70 µl of medium into one port and then add 50 µl into the opposite port of the same media channel to flush out the coating solution. Repeat this for the other channels. If the coating solution has to be removed completely, wash the media channels with culture medium by repeating this step twice.

3 Seeding Cells

- Prepare cell suspensions with densities ranging from 0.5 to 3 M cells/ml, depending on the applications.
- If cells need to be seeded on the gel interface, add an additional 20 µl of medium into one of the ports at the media channel that is to be seeded with cells.
- Position the tip near the media inlet and inject 10 µl of cell suspension into the inlet. Wait for 2 min and then repeat this step at the opposite connected media inlet.
- Visual inspection under a microscope is recommended. If the cell distribution is not optimal for your application, adjust the concentration of the cell suspension and repeat the seeding steps.
- If another cell type B is to be seeded in the opposite media channel, incubate the chip for at least 30 min after cell type A has been seeded to allow proper cell attachment of cell type A on the substrates.

4 Changing Medium

- Remove medium from all 4 ports by carefully aspirating the medium out from the troughs. To change the medium in a media channel, add 70 µl of medium into one port and then add 50 µl into the opposite connected port. Repeat this for the other channel.
- Keep the chips in an incubator. If cells need to be kept longer in culture, change medium **daily** as described in the previous step.