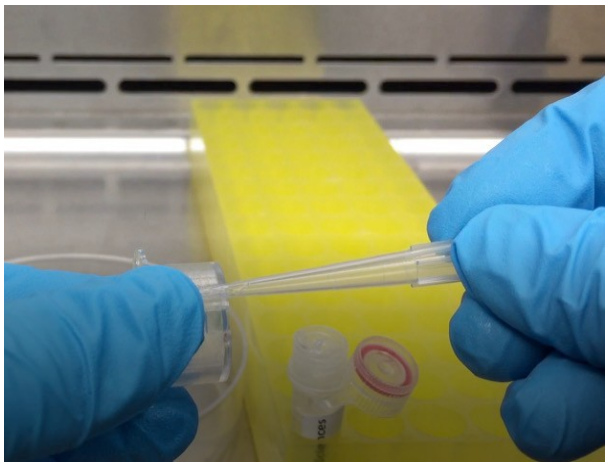
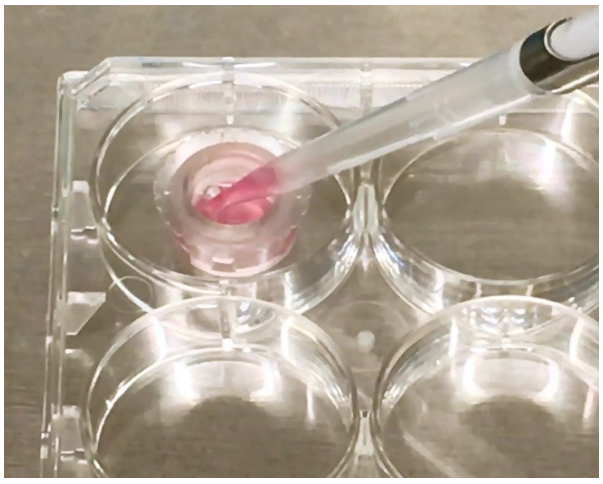
Fennik Life Sciences

Basic procedure to establishing 3D cultures in TheraKan™ devices

1. Lubricate and assemble devices. Insert the inner chamber into the outer chamber so that the openings of the inner and outer chambers are out of frame. This will create a closed system. Place devices in a tissue culture dish, which will served as your test chamber. Recommended test chamber size – 3.5 cm in diameter (6 well plate).

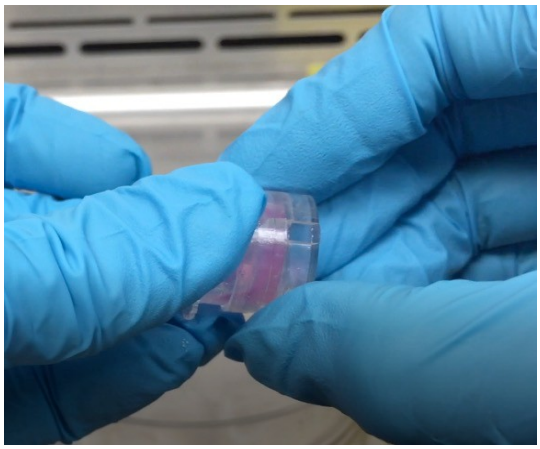


2. Prepare extracellular matrix. Demo shown is with rat tail collagen. This is optional as different protein matrices may be required for each cell type, and would require different steps to prepare. Resuspend the desired number of cells in extracellular matrix. Pipet 275-300 ul extracellular matrix with cells into device. This step would be the same for any protein matrix used. If you are using collagen, let collagen gels polymerize for 10-20 minutes at 37ºC.

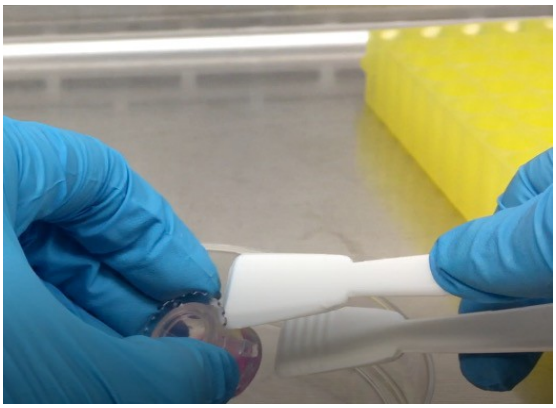


3. Open chamber

a. Method 1: using hands



b. Method 2: using forceps



4. Add media to test chamber. Media should passively diffuse into the devices when opened. You may also add media to devices; we recommend not to exceed 600 ul. Media line should be above the chamber openings.
   Recommendations:
   a. 5 ml ≥ volume of media to a 3.5 cm dish as a test chamber
   b. 10 ml ≥ to 6 cm dish as a test chamber
   c. 10 ml to a 10 cm dish as a test chamber.

Pipet desired number of immune cells into test chamber. Incubate for desired timepoints.

5. To end experiment:

    d. Aspirate media from test chamber.
    e. Process sample, optional: remove sample from device and transfer to Eppendorf tube for fixation or extraction of cells, protein, RNA or DNA

Refer to https://www.fenniklifesciences.com/ for more information and video demonstrations.