Seeding cells in the 3D NanoMatrix™

All handling of the 3D NanoMatrix™ products should be performed using gloves, according to standard aseptic methods.

Pretreatment & Washing

1. Take the Cellevate 3D NanoMatrix™ out of the packaging and place in a biosafety hood. Open the package and inspect the nanofiber scaffold at the bottom of each well. Make sure the lockrings are in place and keeping the scaffolds secure at the bottom.

2. Since the 3D NanoMatrix™ will be incubated in medium or PBS, we suggest pretreating the the fibers by immersion in 70% ethanol. Make sure the liquid volume fully covers the scaffolds.

3. Remove the ethanol solution by tilting the plate and aspirate carefully from the side of the well, being careful not to scratch the scaffolds.

4. After removal of the ethanol, immediately wash each well with PBS two times for approximately one minute.

5. **Optional:** If desired, the NanoMatrix™ scaffolds can now be coated. It's possible to coat the scaffolds with a number of ECM proteins such as Fibronectin, laminin-1, Poly-D/Poly-L-lysine or collagen, using standard protocols supplied by the manufacturer. If you are planning to coat the NanoMatrix™ leave the scaffolds in the second PBS wash until you are ready.

Preincubation & Seeding

1. Before adding your cells, soak the scaffolds with culture media. The volume of culture medium depends on the product format you are using. Make sure to add enough so that the whole scaffold is completely submerged in medium.

2. Place the plate in a humidified incubator for at least 30 min, at 37˚C and 5% CO_2.

3. After preincubation, dissociated cells may now be seeded, or spheroids applied manually, into the scaffolds using standard cell seeding protocols.
   Seed the cells in the middle of the well, and be careful not to touch the scaffold. Since the nanofiber scaffolds provide a much greater surface area than a standard 2D culture plates, the seeding densities should be a bit higher than normal. Initial cell densities of 10^4 - 10^6 cells/cm^2 are suggested. The cell density should of course be chosen based on your cell type and experimental needs.

4. For experiments requiring long incubation times, change media using standard protocols at the normal rates suggested for your cell type.

   Following proliferation, the cells and scaffolds can be used for a wide variety of post-processing applications, including normal protocols for immunocytochemistry, microscopy and *in-vitro* functional studies.

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Recommended seeding densities*

Plate size	Well bottom area (cm²)	Min. cell density	Max. cell density
35 mm dish	9	90 000 cells	9 000 000 cells
60 mm dish	21	210 000 cells	21 000 000 cells
100 mm dish	55	550 000 cells	55 000 000 cells
4-well	1.9	19 000 cell/well	1 900 000 cells/well
6-well	9.5	95 000 cells/well	9 500 000 cells/well
12-well	3.8	38 000 cells/well	3 800 000 cells/well
24-well	1.9	19 000 cells/well	1 900 000 cells/well
48-well	0.95	9500 cells/well	950 000 cells/well
96-well	0.35	3500 cells/well	350 000 cells/well

*Suggested densities, please adjust according to your experimental needs.

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