

## Seeding cells in the 3D NanoMatrix™

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

Take the Cellevate 3D NanoMatrix<sup>™</sup> 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 keep the scaffolds secure at the bottom.

## Optional Coating Step:

The NanoMatrix<sup>™</sup> scaffolds can be coated with different extracellular matrix components such as fibronectin, laminin, collagen, vitronectin, and others, following protocols supplied by the manufacturer. The NanoMatrix<sup>™</sup> scaffolds can also be coated with Poly-D/Poly-L-lysine. If you are planning to coat the NanoMatrix<sup>™</sup> wash the scaffolds once with PBS and proceed with coating substrate manufacturers instructions. After coating, proceed to the cell seeding step described below.

## Preincubation Step:

Soak the nanofiber scaffold with culture media and place the plate in a humidified incubator for at least 30 min (e.g. at 37°C and 5% CO<sub>2</sub>). The volume of the culture medium depends on the product type and format you are using. Make sure to add enough so that the whole scaffold is completely submerged in medium.

# Cell Seeding Step:

Seed the cell suspension into the scaffolds using standard cell seeding protocols.

Note: 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 plate, it is recommended to seed at higher densities than in 2D. Initial cell densities of 104 - 106 cells/cm² are suggested. The cell density should of course be chosen based on your cell type and experimental needs.

## Media Change Step:

Change media using standard protocols at the normal rates suggested for your cell type.

# Downstream Applications:

Following proliferation, the cells and scaffolds can be used for a wide variety of post-processing applications (e.g. immunocytochemistry and microscopy, Cell recovery, DNA/RNA/protein), various in vitro cellular assays (e.g. viability, proliferation, migration)