



Cellevate

Seeding cells.*

> Precondition & Washing

1. Take the Cellevate 3D™ plate / dish out of the packaging and place in a biosafety hood.
2. Wash the plate 2 times using PBS or sterile water. Aspirate carefully from the edge of the insert, being careful not to touch the scaffold.
3. **Optional:** Some cell types can have low attachment to plastic (neural and tumor stem cells). If desired, nanofiber scaffolds can now be coated with suitable ECM protein, e.x. fibronectin (5-10 µg/ml) or laminin-1 (5- 20 µg/ml) using protocols supplied by the coating manufacturer. (See the protocol "*Laminin coating of nanofiber scaffolds*" for suggestion).
4. Before adding your cells, we suggest soaking the fibers with sterile culture media, followed by incubation for at least 30 min, at 37°C. After preincubation, rinse the scaffolds twice with PBS and 1x with desired cell culture medium.

> Seeding

1. Cells may now be dissociated and seeded, or spheroids applied manually, into the scaffolds using standard protocols.
Note: Seeding densities of 10^4 - 10^6 cells/cm² are suggested (see "*Recommended seeding densities*" table on the next page), but change this to suit your specific cell type and your experimental needs accordingly.
2. For experiments requiring long incubation times, change media at the normal rates suggested by media supplier.

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



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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 cells / 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
384 - well	0.1	1000 cells / well	100 000 cells / well

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