

## Usage of Microcavity Array Plates:

### Deaeration of the microcavity array:

Use an alcohol series of isopropanol with the following steps: 100%, 70%, 50%, 30%, PBS.

- Pipet 200 µl of solution into each well to be seeded with cells by revers pipetting the solution three to five times into the well. Try to wash out obvious bubbles in the microcavities.
- Afterwards, remove the solution by turning over the plate and tapping it on a soft cloth
- Do that for all steps of the alcohol series and then finally for PBS.
- Try to remove excess fluid from the well area of the plates with only 10 microcavities by using a Pasteur pipette and vacuum pump device for example. This will lead to a better focusing of the cell pellet above the microcavity area.
- Once this has been done, don't let the plate get dry, otherwise the procedure has to be repeated.

### Collagen coating (not for low attachment plates!)

- Prepare a stock solution of 2 mg/ml collagen I in 0.1% acetic acid. From this stock solution mix 4.5 µl + 33 µl sterile water for coating of one well. Pipet the solution into the well and incubate at 4°C over night (4 h at least).
- Afterwards, wash three times with PBS using the technique explained under deaeration.

### Inoculation of cells:

- For e.g. cell lines, such as Hep G2, use 0.5 to 1 Mio cells/well of a 96-well plate with 169 microcavities, depending on your experimental purpose.

For a plate with 10 microcavities use 30.000 to 40.000 cells in a drop of 3 to 4 µl of medium that is positioned directly on top of the microcavities. The cells will sediment into the microcavities by gravity.

- The process can be facilitated by centrifugation up to 50 g prior to incubation.

- Alternatively, use an Orbital shaker with around 40 rpm or a Horizontal Shaker with 250 rpm for 2 min.
- After the inoculation, cover the plate with the lid and incubate at standard conditions for 15 to 30 min before adding up to 100 µl of culture medium.
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We recommend to culture the cells 24 hours in the plate prior to the experiment.

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