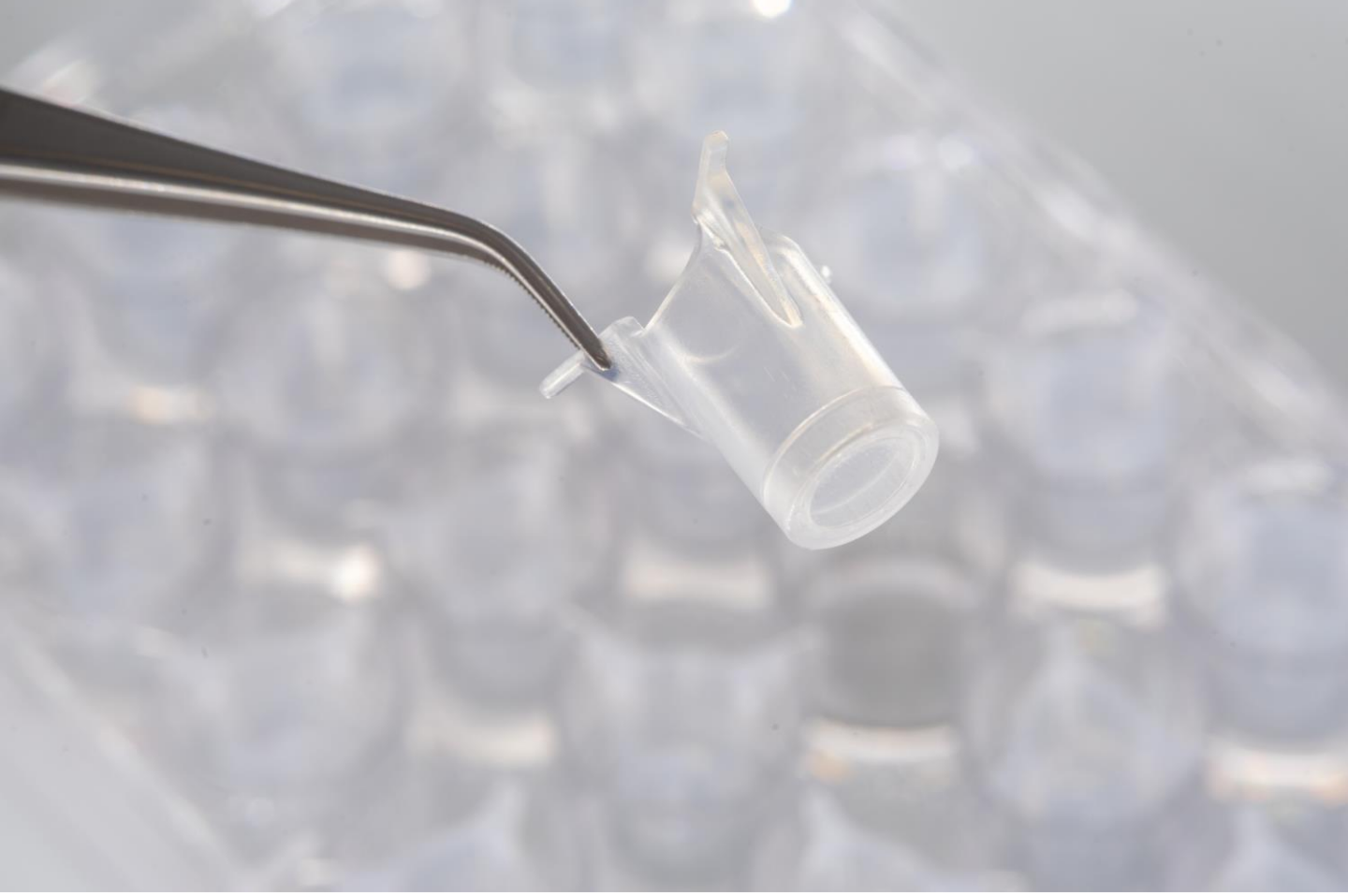


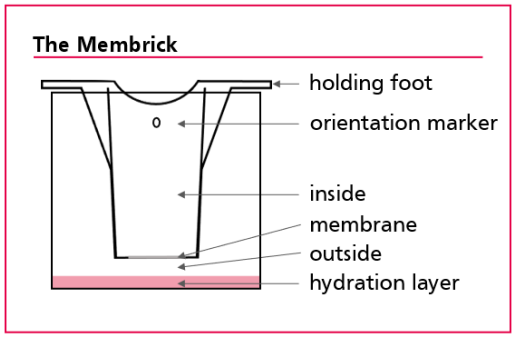
Membrick

User Manual

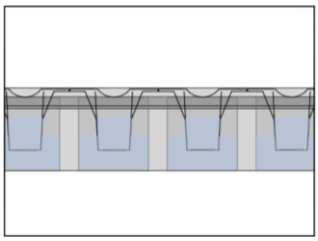
CELLBRICKS

The Membrick is a 24-well multiple well plate-compatible cell culture platform. It combines the ease-of-use of conventional cell culture inserts (e.g. Transwell®) with the capability to create completely biological barrier models. Handling the Membrick e.g. for seeding cells or replacing culture medium is similar to handling conventional cell culture inserts.

Table of Contents	Page

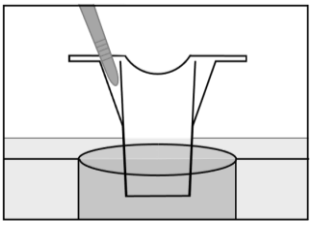


1. Receive & Store



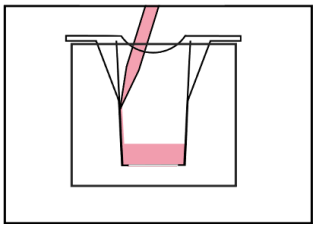
The Membrick is shipped in a sterile 24-well plate with a hydrogel-based hydration layer to ensure a humid environment. After receiving the Membrick, addition of a storage buffer is required to prevent dehydration, if the Membrick is not used immediately upon receipt. Move the inserts to the empty wells in row A and D, and add 1000 µL sterile PBS to the wells and 200 µL inside the insert. Seal the plate with Parafilm and store at 4°C.

2. Moving the Membrick



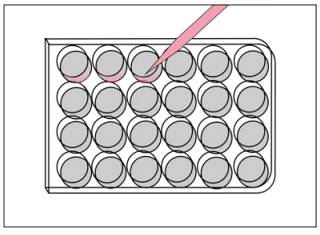
The Membrick hangs in the well of a 24-well plate with the three feet resting safely on the well rim. Moving the Membrick is done by gripping its feet with forceps.

3. Add medium internally



Culture medium volumes are 150 µL inside the Membrick. For adding medium to the Membrick, use a 200 µL pipette and gently let the medium flow down along in the inner wall of the Membrick.
CAUTION: Avoid pipetting directly onto the membrane.

4. Add medium externally



For removing and adding medium from/to the well, use a 1000 µL pipette.
Hints: Tilt the plate slightly to ease aspiration of all the liquid. Moving the Membricks to an adjacent well can help provide access to the well.

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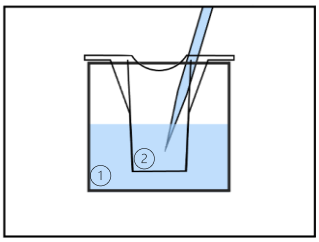
Required materials

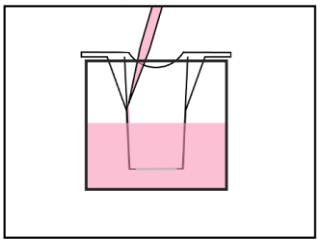
- Cell suspension with desired cell type(s)
- Sterile 24-well ultra-low attachment plate containing required number of Membricks

1. Remove storage buffer



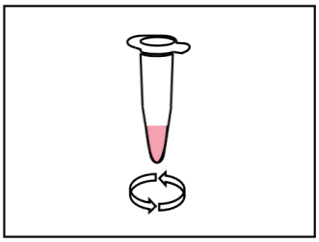
Remove any storage buffer first from outside (1) and then from the inside (2). Use the plastic tip rest on the inner side to safely place the pipette tip. For this, cautiously insert the pipette tip in the Membrick body and slowly slide down along the inner wall until you hit the tip rest. Gently aspirate all the medium. Place the Membrick back in the well.

2. Add medium



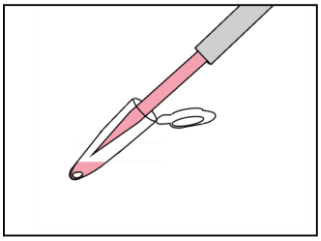
Gently add 150 µL cell culture media to the inner space and 650 µL outer space of Membrick. Gently let it flow down along in the inner wall of the Membrick. Incubate the Membrick at least 1h in your cell culture media.
CAUTION: Avoid pipetting directly onto the membrane.

3. Centrifuge cells



Add the desired number of cells to a sterile reaction tube and centrifuge the cells as usual. Remove as much of the supernatant as possible. The appropriate number of cells will depend on the cell type and experiment. *10,000 cells/Membrick is recommended as a starting point*.

4. Resuspend cells



It is recommended to resuspend the pellet in a volume of 100 µL of the appropriate medium per Membrick (0.32 cm^2) to be seeded.

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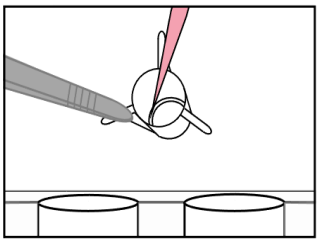
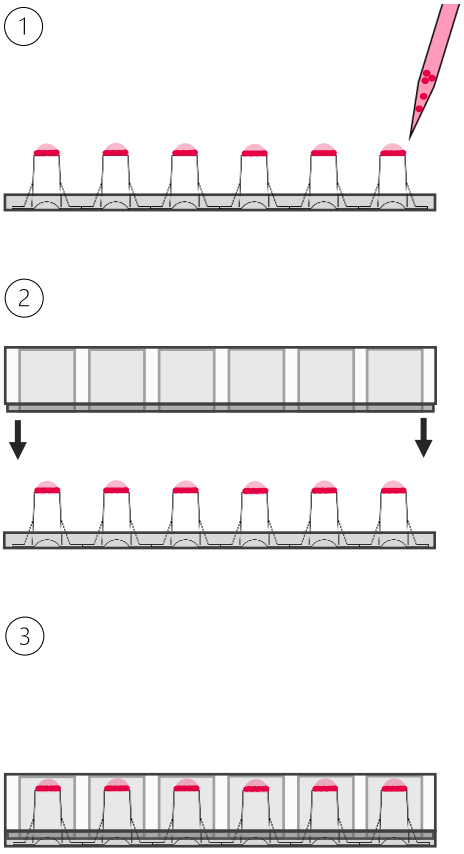
It is possible to seed cells on both sides of the membrane. If you only want to seed cells inside the Membrick, skip points 5 & 6.

5. Remove medium



5.1 Remove the medium from the well.
5.2 Remove Membrick from the well and remove the medium from the inside of the Membrick. Use a 200 µL pipette. Use the side of the well and the plastic tip rest to avoid touching the membrane with the pipette tip.
5.3 Place the Membrick upside down on the lid of the 24-well plate. Ensure the Membrick is aligned with the location of its corresponding well. On many plates these locations are indicated on the lid with circles.

6. Seeding cells on the <u>outer</u> side of the Membrick



6.1 Once you have placed the Membricks in the correct position, pipette **40** µL of the cell suspension slowly and carefully onto the membrane. Ensure the entire surface of the membrane is covered, and that the pipette tip does not touch the membrane.
6.2 After seeding is done, very carefully place the bottom of the plate over the Membricks to cover them, and avoid touching the seeded membrane. Move the plate cautiously in a figure-8 motion to improve distribution of the cells. Keeping the plate in the inverted orientation (i.e. closed with lid underneath) place the plate with the Membricks in the incubator for at least 2 h, until the cells have attached. This will vary by cell type.
6.3 Take plate out of the incubator and in a single movement slowly flip the plate. Ensure the plate remains closed during this process. Add 650 µL of medium to the well outside the Membrick. Then add 150 µL of medium inside the Membrick (you can skip the addition inside the Membrick if you will proceed immediately to the next step).

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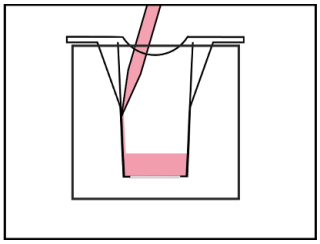
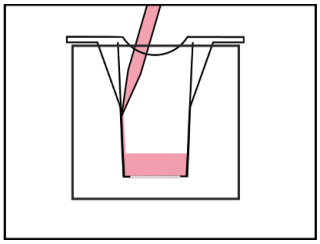
7. **Seeding cells on the inner side of the Membrick**
Pipette 100 µL of the cell suspension cautiously into the Membrick. For an optimal distribution of the cells, pipette the cell suspension into the Membrick in a circular movement, avoiding pipetting directly into the walls of the Membrick. Finally move plate in a figure-8 shape. Place the plate with the Membricks in the incubator and incubate (1-2h) for cell attachment.



CAUTION: be sure not to disturb the freshly settled cells as their adhesion to the biological membrane might still be weak.

As a starting point, we recommend seeding 10^4 cells per side of the Membrick (optimal seeding density may vary depending on cell type).

8. **Optional: Co-culture with a third cell type**
If you wish to cultivate with an additional cell type growing on the bottom of the well, prepare these cells in culture on a separate plate. At the point you wish to begin co-culture, move the Membricks (already seeded as per previous steps) into this well-plate, and adjust the medium volumes to 150 µL inside the brick, and 650 µL in the well outside the brick.



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