FakirSlide

Protocol

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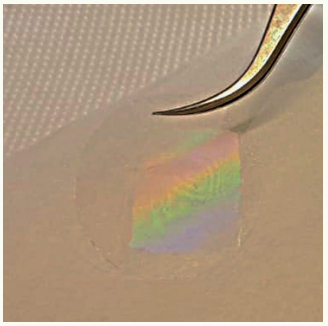
Before you start: how to place a Stencell on FakirSlide coverslips

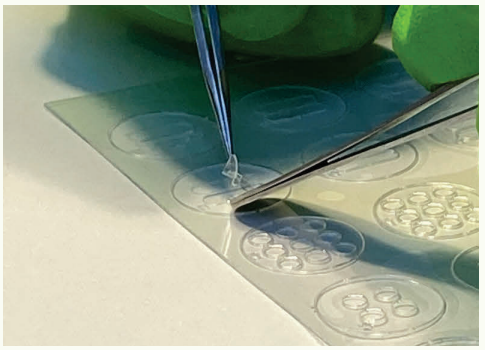
The nanostructures present on the coverslip create a hydrophobic surface. Defining restricted coating area allows to maximize surface wetting and coating homogeneity while saving reagents. For this aim, we recommend using the Stencell chambers provided with the kit. Any other equivalent cell culture inserts can be used instead.

- Delicately place the FakirSlide coverslip in the culture vessel of your choice using tweezers.



! Avoid touching the patterned area with the tweezers.

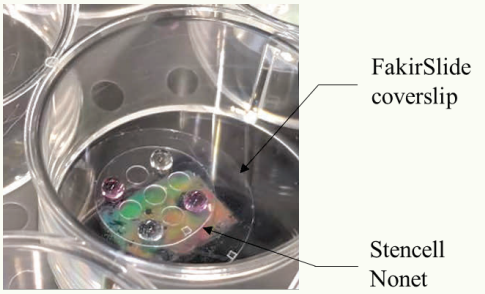
- Using tweezers, remove the protective layer on the Stencell.
- Gently remove the inner elements present on the Stencell design.



Use 2 tweezers to press close to the junction in order to avoid breaking the Stencell. Alternatively, junctions can be cut with a scalpel.

- Remove the Stencell from the lower adhesive layer.
- Place it on top of your FakirSlide coverslip to define your cell culture area(s).

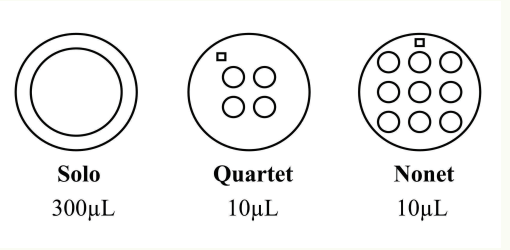
Stencells can be placed at the interface between structured & non-structured areas to include a non-structured internal control.



! Press delicately on the Stencell to avoid breaking the coverslip.

- Gently remove the bubbles and stick the Stencell, patting it with tweezers.

Excess volumes of liquid need to be loaded in the Stencell chambers to properly fill the whole surface area. Recommended volumes to be loaded per chamber for each Stencell design are listed in the picture below.



! Do not overfill the wells to prevent liquids from leaking inbetween chambers.

I. Cell culture on FakirSlide coverslips

1. Important points before starting

- The coverslips provided are 25mm large, but the patterned surface is smaller than total coverslip area. If the coverslip does not match your culture vessel dimensions, it is possible to cut it around the patterned surface using a diamond engraving pen.

- As the nanostructures present on the FakirSlide coverslips create a hydrophobic area, we strongly recommend coating the cell culture area with adhesive proteins to guarantee optimal cell adhesion and spreading on structures.

- The choice of optimal coating solution will vary depending on your cell characteristics and experimental goals. The following combinations have been successfully used on the FakirSlide coverslips (non-exhaustive list):

Cell type	Poly-L-Lysine 0.01%*	Fibronectin 50µg/mL**
HeLa	Validated	Validated
U-2 OS	Difficult adhesion	Validated
HT1080	Validated	Not tested
C2C12	Validated	Validated
SUM159	Validated	Validated
RPE-1	Not tested	Validated
THP-1	Validated	Validated
moDC	Validated	Validated

Table 1. List of coating solutions previously validated on FakirSlide coverslips for given cell types

*The Poly-L-Lysine 0.01% coating solution can be made in sterile water from a 1mg/mL stock solution resuspended in $NaHCO_3$.

**The Fibronectin 50µg/mL coating solution can be made in sterile water from a 0.1% stock solution (Sigma-Aldrich, Cat#P8920). It can also be ordered already diluted at the final concentration (Sigma-Aldrich, Cat#P4707).

2. The material you need

Reagents

- Coating solution (i.e. Poly-L-Lysine, fibronectin or others)
- Sterile DPBS
- Sterile water
- Culture medium

Consumables

- FakirSlide coverslips (provided in the kit)
- Stencell silicon chambers (provided in the kit)
- Culture vessel of your choice (i.e. 6-well plates)
- Tweezers

3. Coating your FakirSlide coverslip

- Prepare your FakirSlide coverslip and Stencell chambers according to the protocol described in page 1.

- Wash your coating area by adding sterile water and pipetting up and down several times.

- Add your coating solution to the coating area using the volumes recommended in Table 2.

- Incubate at 37°C/5%CO_2 for 1h.

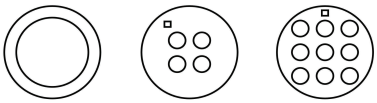
4. Cell seeding

• Detach your cells and resuspend them in appropriate cell culture medium at the desired cell density.

Optimal seeding density may be adjusted depending on your cell type and targeted application. As a general rule, we recommend seeding cells at a low density to allow proper spreading of cells on the nanostructures. As a reference, HT1080 cells are typically seeded at a density of 180,000 cells in one Solo Stencell and 6,000 cells per chamber in the Quartet & Nonet Stencells when assayed the day after.

• Wash the seeding area twice with sterile DPBS, then once with culture medium.

• Add your cell suspension to the coated areas using the volumes recommended below.



Solo	**Quartet**	**Nonet**
300µL	10µL	10µL

• Place your culture back in the incubator and leave cells to adhere.

Most cell types should adhere to the nanostructures after ~4h, but leaving them overnight will guarantee maximal spreading on the structures.

! Do not incubate more than 12 hours without renewing the culture medium.

• Once cells adhered, fill the entire culture vessel with culture medium to initiate long-term culture. If needed, Stencell silicon chambers can be removed for the culture vessel at that stage.

• Proceed with your usual protocols for cell culture, live cell imaging or immunostaining.

II. Using FakirSlide for supported membranes

1. Important points before starting

- The FakirSlide coverslips can be used as substrates for supported lipid bilayers *in vitro*.

- This protocol might be adjusted depending on your experimental settings and usual protocols (i.e. unilamellar vesicle preparation method, saline buffer composition, etc). As a general rule, any protocol that has been optimized on classical flat glass substrates might be applied to the FakirSlide coverslips.

2. The material you need

Reagents

- Suspension of unilamellar vesicles (lipid mixture of your choice)
- Sterile milliQ water

Consumables

- FakirSlide coverslips (provided in the kit)
- Stencell silicon chambers (provided in the kit)

Others

- Attofluor™ Cell Chamber (or equivalent) for microscopy

3. Preparation of unilamellar vesicles

Unilamellar vesicles made of a lipid mixture of your choice can be prepared using a classic extrusion method. The choice of saline buffer to use will depend on your lipid mixture composition. The following saline buffers have been validated:

Generic lipid mixture	Saline buffers
100% mol neutral lipid(s)*	Standard saline buffers (i.e. PBS or 20mM Tris pH 7.4, 150mM NaCl)
80% mol of neutral lipid(s) / 20% mol negatively-charged lipid(s)**	Citrate 20mM pH 4.6, KCl 50mM, EGTA 0.5mM

Table 2. List of saline buffers previously validated for given lipid mixture compositions

*Examples of neutral lipids tested include DOPC, Egg-PC, POPC, POPE & Egg-PE

**Examples of negatively-charged lipids tested include Liver-PI, Brain-PS, Brain-PI(4,5)P2 & Brain-PI4P

0.2% mol of fluorescent lipid analogs (i.e. OG-v) can be added to the lipid mixture for the visualization of membrane topography.

4. Loading the supported membranes on FakirSlide

- Place your FakirSlide coverslip in the Attofluor™ Cell Chamber or equivalent

- Prepare your FakirSlide coverslip and Stencell chambers according to the protocol described in page 1.

- Wash your coverslip twice with sterile milliQ water

- Add your vesicle suspension to the pre-cleaned coverslip using the volumes recommended in Table 2.

We recommend using a 0.25 mM unilamellar vesicle suspension to properly cover the coverslip surface with isolated membranes.

- Place your coverslips in a oven at an appropriate temperature for 40 min.

Optimal temperature will depend on your lipid mixture and should be above its transition temperature.

Using a humid chamber can help prevent vesicle suspensions from drying out during the incubation time.

- Wash the coverslips five times with your buffer of choice.

- Proceed with your usual protocols for isolated membrane imaging.

Check our website for example results, updated FAQ and much more

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Publishing a study using FakirSlide? Please cite their original publication:

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