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Coverslip Functionalization SOP003.v2.2 V.1

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protocol .

Human Cell Atlas Method Development Community

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Document Summary:
This document, SOP003 – Coverslip Functionalization, describes the process for cleaning and modifying the coverslip surface for improved adhesion of a sample.This procedure describes two methods of functionalization, silanization and poly-d-lysine (PDL) coating. Silanization is a method used to promote covalent adhesion of polyacrylamide gel-embedded tissue while poly-d-lysine (PDL) coating of silanized coverslips provides a protein anchoring system which improves adhesion of living cells to the surface of the coverslip.

Quick Overview:
Part 1 - Clean coverslips
Part 2 - Silanize coverslips
Part 3 - PDL coat coverslips

Coverslip Functionalization SOP003.v2.2.pdf

Rory Kruithoff, Douglas Shepherd 2021. Coverslip Functionalization SOP003.v2.2. **protocols.io** https://protocols.io/view/coverslip-functionalization-sop003-v2-2-byiipuce

Coverslip, silanization, poly-d-lysine, coverslip cleaning, tissue adhesion, cell adhesion, allyltrichlorosilane

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v2.2 revision notes
-Added Bottiger lab and OsmFISH references.

References:

Moffitt, J. R., Hao, J., Bambah-Mukku, D., Lu, T., Dulac, C., & Zhuang, X. (2016). High-performance multiplexed fluorescence in situ hybridization in culture and tissue with matrix imprinting and clearing. *Proceedings of the National Academy of Sciences*, *113*(50), 14456-14461. https://doi.org/10.1073/pnas.1617699113

Boettiger Lab Github:
https://github.com/BoettigerLab/protocols/blob/master/MatrixEmbedding.md

OsmFISH dx.doi.org/10.17504/protocols.io.psednbe

Solution Preparation:

Silanization Solution

- [M]**0.1 % (v/v) triethylamine** (Millipore, TX1200)
- [M]**0.2 % (v/v) allyltrichlorosilane** (
  Allyltrichlorsilan **Sigma**
  Aldrich Catalog #107778)
  Chloroform **Sigma**
- dissolved in chloroform (**Aldrich Catalog #C2432-1L**)

PDL (Poly-D-Lysine) Solution

- [M]**0.1 mg/mL Poly-D-Lysine** (molecular weight 30,000-70,000 Da;
  Poly-D-lysine hydrobromide (PDL) **Sigma**
  Aldrich Catalog #P7886)
- Dissolved in nuclease-free water.

Additional Reagents:

Ethanol **Fisher**

- **Scientific Catalog #AC615090010**
  Chloroform **Sigma**
- **Aldrich Catalog #C2432-1L**
  Methanol **Sigma**
- **Aldrich Catalog #34860-1L-R**
  Hydrochloric acid **Sigma –**
- **Aldrich Catalog #320331-500ML**

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Using 40-mm-diameter #1.5 coverslips

Part 1 - Clean coverslips 30m

30m

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Wash for **00:30:00** via immersion in 1:1 mix of [M]**37 % (v/v) HCl** and methanol at **Room temperature** .

2

Rinse 3x in DI H2O.

3 Fill beaker with Rnase-free water and autoclave coverslips to sterilize.

4 

Rinse 1x in [M]**70 % Ethanol** .

5 Coverslips can be stored in [M]**70 % EtoH** indefinitely.

Part 2 - Silanize coverslips

6 Dry at **60 °C** .

7 Immerse in silanization solution in Fume-hood for **00:30:00** at **Room temperature** . 30m

8 

Wash 1x with chloroform.

9 

Wash 1x with ethanol.

10 Bake in a **60 °C** oven for **02:00:00** to dehydrate the silane layer. 2h

11 Remove from oven and let coverslips cool.

12 Store in a desiccated chamber for weeks.

Part 3 - PDL coat coverslips (for cell culture on coverslips) 1h

1h

13 Immerse coverslips in PDL Solution for **01:00:00** at **Room temperature** .

14 

Rinse coverslips three times with nuclease-free water and then dry at **Room temperature** .

15 Silanized, PDL-coated coverslips may be stored at **Room temperature** in a desiccation chamber for weeks.