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Single-Molecule Antibody Slides For Fluorescence Microscopy

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We use this protocol and it's working

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Abstract

This protocol describes how to create single-moelcule antibody slides for fluorescence microscopy.

Troubleshooting

Coverslip Cleaning

1h 0m 10s

1 Coverslips (24 × 50 mm, #1, VWR,Catalogue Number 48404-453) were argon plasma cleaned (Ar plasma cleaner, PDC-002, Harrick Plasma) for 00:30:00 . 30m

Surface preparation

1h 0m 10s

2 A trimmed gasket was placed on top of the slide (CultureWell™ Reusable Gasket, 6mm diameter, Grace Bio-Labs, SKU: 103280).

3 Poly-L-Lysine 30 µL (0.01 % w/v PLL, P4707, Sigma-Aldrich) was placed in the wells for 00:30:00 . 30m

4 The PLL was removed, the wells washed three times with PBS (pH 7.4, 1x Gibco, Thermo Fisher Scientific, Catalogue number 10010023)

Depositing Antibodies

1h 0m 10s

5 A secondary antibody of choice was added at a final concentration of 0.0002 mg/ml in PBS.

6 The antibodies were left in the wells for 5-10 seconds for sufficient surface density before the wells were washed three times with PBS. 10s

7 PBS 30 µL was left in the wells for imaging