Glass Coverslips Preparation for Magnetic Tweezers

Bottom Surface Cleaning

1. Sonicate surfaces for 30 min in detergent Hellmex lll 1% (50 °C)
2. Wash with DD water 10x
3. Sonicate with acetone for 30 min
4. Wash with DD water 10x
5. Wash with ethanol molecuLar biology
6. Sonicate with ethanol for 30 min
7. Remove ethanol dry with air duster or compressed air

~The "superslips" can be left in ethanol overnight if necessary.

Bottom Surface Silanization

1. Silanization with 1% silane (1,5mL silane in 150mL EtOH or MeOH) for 20 min
2. Wash with EtOH (MeOH) 3 times and dry with air duster or compressed air
3. Bake for >1hrs at 100 °C
4. Store in drying stove or desiccator

~The protocol can be stopped here and continued later. The glasses can be stored in a petri dish

Cover Surface Cleaning

1. Sonicate surfaces for 10 min in detergent 1%
2. Wash with DD water 10x
3. Wash with ethanol
4. Dry in the oven for ~10 min

~The protocol can be stopped here and continued later. The glasses can be stored in a petri dish

Fluid cell

1. Cut 6x40 mm parafilm strips.
2. Put on a hotplate at 90 °C and push the sandwiches to melt the parafilm
3. With the hydrophobic pencil make lines in the cameras

~Be sure that the chamber is well fixed between both covers; the protocol can be stopped here and continued later. The glasses can be stored in a petri dish

Surface Preparation

1. Fill fluid cells with 1% Glutaraldehyde solution (PBS; 20 µL 70% in 1.3 mL) and let it react for 1h
2. Add 100 µL 200x dilution of reference beads (10 µL in 1.2 mL PBS): no washing before!; leave for 10-20 min; check the beads adsorption with a microscope
3. Add 100 µL 500x diluted Halo ligand (10 µg/mL; 1.3 mL PBS + 8,4 µL Halo 5mg/mL) and left to react **overnight**

4. Wash 3x 100 µLTRIS/BSA 1%.Leave with 200µL TRIS/BSA 1% to passivate **overnight**

~The protocol can be stopped here and continued later; but after the time is necessary to wash 3x 100 µL HEPES/BSA 1% and then the microfluidic chambers can be stored for approx. 2 weeks in a humidity chamber at 4°C

5. Wash 3x with 200 µL HEPES/BSA
6. Dilute the HaloBiotin protein 1-10 µM in HEPES/BSA 1% (1000x - 10,000x) Add 200µL of the protein and left to react for 20 min
7. Lift magnet and add 60 µL streptavidin beads (30µL streptavidin beads + 1mL HEPES/BSA 1%; wash 3x and separate with a magnet; the beads are removed from the solution: leave overnight in the rotator)

~To store the chamber, wash 3x with 200 µL HEPES/BSA, and then leave it with 200 µL HEPES/BSA in a humidity chamber at 4°C. To continue in another experiment remove the excess

Materials

- Coverslips 22x22 mm 0.13-0. 16 mm thickness, TED Pella, N260140
- Coverslips 24x40 mm 0.13-0. 16 mm thickness, TED Pella, N260164
- Hellmanex (1%), Hellma, Z805939
- APTES, Sigma Aldrich, 281778-5ML
- Glutaraldehyde, Sigma Aldrich, G7776-10mL (-20C)
- Amino-polystyrene Beads 2.5-2.9 um, Spherotech, AP-25-10
- HaloTag 04 amine ligand 5mg/mL in DMF/DMSO, Promega, P6741 (-80C)
- Dynabeads M-270 Streptavidin, Invitrogen, 65305
- BSA sµLfhydryl blocked, Lee BioSolutions, 100-10SB

Buffers

PBS

10 mM	Phosphate buffer pH 7.4
137 mM	NaCl
27 mM	KCl

Tris buffer

20 mM	Tris-HCl pH 7.4
150 mM	NaCl
2 mM	MgCl2
1%	BSA thiol blocked
0.01%	NaN3 (Only if is available in the lab)

Hepes buffer

20 mM	Hepes pH 7.2
150 mM	NaCl
1 mM	EDTA