

Protocol 2: Preparation of Coverslips.

This protocol takes 2-3 hours and will prepare 24 coverslips.

You will need:

No.1 glass coverslips (22x22 or 22x30 mm)

2 ceramic coverslip holders for 12 coverslips each (cat# 8542e40, Thomas Scientific)

6 washing jars, each will hold 4 coverslips stacked together (cat# 21036, Ted Pella, Inc.)

two 500-ml beakers

four 250 ml glass jars (6 cm in diameter, so it can fit coverslip ceramic holders)

Molecular Sieves Grade 564 3 Å (cat# M564-500, Fisher Sci)

500 ml of PlusOne Repel Silane solution (Silane A174 (Repel-Silane ES), GE Healthcare Life Sciences, 17-1332-01).

Plasma cleaner model (cat#, PDC-32G Harrick Plasma)

Forceps (cat# H37942-0000, Thomas Scientific)

methanol

All following steps should be carried out in a fume hood, while wearing gloves.

2.1. Rinse coverslips in ethanol and dry with KimWipes. Stack the coverslips in ceramic holders (12 coverslips per holder). While preparing step 2.2 keep the ceramic holders covered empty 250 ml glass jars to avoid dust from sticking to the coverslip surface.

2.2. Activate Molecular Sieves by heating at 200°C for 1 h. Wait for Molecular Sieves to cool down to room temperature while keeping inside the switched-off oven (to avoid water absorption from the air). Cover the bottom of two 250 ml glass jars (6 cm in diameter) with Molecular Sieves for water absorption.

Fill the jars with 200 ml of Silane solution. **Warning:** Do not add PlusOne Repel silane directly to Molecular Sieves until they cool down to room temperature because PlusOne Repel Silane is flammable!

2.3. Plasma Clean coverslips (up to 2 ceramic holders simultaneously) for 15 min at 30 W. Pressure inside the cleaning chamber is set at 800–950 mTorr. Both atmospheric and compressed oxygen can be used.

2.4. Immediately after Plasma Clean slowly immerse the ceramic holders with coverslips in the jars, close the lids and incubate for 5 min at room temperature. This will create hydrophobic coating on the coverslip surface.

2.5. Slowly remove the holders with coverslips from the jars and transfer them to 500 ml beaker filled with 200 ml methanol to fully submerge all coverslips.

2.6. Place a metal or glass pedestal into the water reservoir of a sonic bath, such that the liquid in the sonication bath and in the beaker are at the same level. Sonicate at 70 W for 20 min, changing methanol solution every 5 min. Do not drain methanol into sink, dispose into designated container, for later pick-up by EHRS.

2.7. Place coverslips into the 6 washing jars (4 coverslips each), rinse coverslips 30 times with milli-Q water. If silanization worked properly, the coverslips will appear dry when removed from water. Thoroughly remove any residual water using nitrogen if needed.

2.8. Interlay the coverslips with KimWipes to avoid surface-to-surface contact between the coverslips. Coverslips can be stored in a sealed container for several weeks at room temperature.