

Protocol 2: Preparation of Coverslips.

This protocol takes 6-8 hours and will help to prepare 36 coverslips. You will need 3 ceramic coverslip holders and 9 coverslip staining jars with lids; jar volume should be 15 mL, so each will hold 4 coverslips stacked together. Use regular No.1 glass coverslips (22x22 or 22x30 mm). All steps should be carried out in a fume hood, while wearing gloves.

2.1. Put the coverslips into the glass coverslip staining jars and fill the jars with acetone. Incubate for 1 hr, wash 10 times with deionized water

2.2. Incubate the coverslips 10 min with ethanol and wash again 10 times with deionized water.

2.3. Prepare “piranha” solution. Put 60 mL of hydrogen peroxide solution (30% in water) in a heat-resistant glass vessel and slowly add 100 mL of sulfuric acid (final ratio of acid to hydrogen peroxide solution is 5:3). Solution will heat up, this is normal but use caution. **Piranha solution is extremely corrosive! Use thick lab coat, gloves and goggles!**

2.4. Fill the coverslip staining jars with “piranha” solution, close the lids and place the jars in a water bath preheated to 90°C for 1 hr.

2.5. Pour off the “piranha” solution and discard as instructed by the safety regulations at your workplace. Wash coverslips 10 times with deionized water.

2.6. Fill the coverslip staining jars with 0.1 M KOH, incubate 10 min, and wash 10 times with deionized water. This will neutralize any acid residues left on the coverslips after “piranha” treatment.

2.7. Dry coverslips one at a time by holding each coverslip with Teflon coated flat-edged tweezers (to minimize damage to a glass surface) and while blowing compressed dry nitrogen. Make sure that coverslips are dried completely, because silane solution is highly reactive with water.

2.8. Stack the dried coverslips in ceramic holders (12 coverslips per holder), which should be thoroughly pre-dried with nitrogen. Keep the ceramic holders covered to avoid dust from sticking to the coverslip surface.

2.9. Cover the bottom of 250 mL glass jar (6 cm in diameter) with Molecular Sieves, Grade 564, for water absorption.

2.10. Fill the jar with 200 mL of PlusOne Repel Silane solution and slowly immerse a ceramic holder with coverslips in a jar, close the lid and incubate for 5 min at room temperature. This will create hydrophobic coating on the coverslip surface.

2.11. Slowly remove the holder with coverslips from the jar and transfer coverslips one at a time into the coverslip staining jars filled with methanol.

2.12. Place a metal or glass pedestal into the water reservoir of a sonic bath, such that the coverslip staining jar is immersed for 2/3 of its height. Sonicate at 70 W for 20 min, changing methanol solution every 5 min, then rinse 10 times with deionized water. If the silanization worked properly, the coverslips will appear dry when removed from water.

2.13. Thoroughly remove any residual water using nitrogen, as above.

2.14. 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.

Note 1. Steps 2.1-2.6 can be replaced by cleaning the coverslips with Plasma Cleaner for 15 min at 30 W, greatly reducing the total preparation time. Pressure inside the cleaning chamber is set at 100-200 mTorr. Both atmospheric and compressed oxygen can be used. Stack the plasma-cleaned coverslips in ceramic holders and proceed to step 2.7.