Glass Slides and Coverslips Preparation for Microscopy

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This protocol was adapted from Chada, N. et al., '*Glass is a Viable Substrate for Precision Force Microscopy of Membrane Proteins*'. *Scientific Reports* **5**, 12550, doi:10.1038/srep12550 (2015). https://doi.org/10.1038/srep12550

INTRODUCTION

It is important that the glass slides and coverslips used in microscopy experiments be extremely clean. Although commercially available coverslips and slides look clean, especially when out of a new box, they may have a thin film of grease and other contaminations on them. Therefore, coverslips and glass slides should be routinely treated with acid or base solutions to get rid of these contaminants. This protocol describes an easy approach for cleaning glass slides and coverslips and sterilizing them for various microscopy applications.

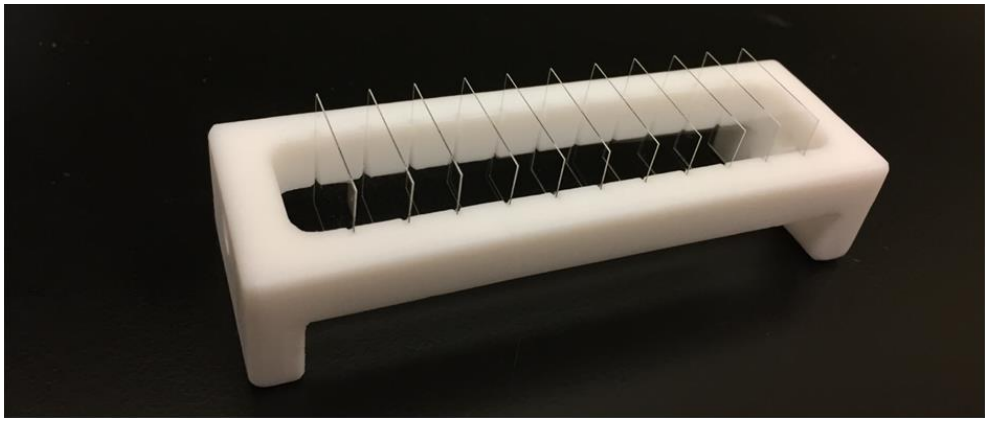
CHEMICALS, EQUIPMENT & VENDORS

Glass coverslips: Corning (18 × 18 mm, No. 1.5, catalog #: 2850-18)
KOH pellets (Sigma Aldrich, catalog #: P5958)
Absolute ethanol (Fisher Scientific, catalog #: BP2818)
Sonicator (Branson 5510)
Magnetic stir plate and stir bar
DI water and three sterilized 1L beakers
Nitrogen or Argon gas to blow dry coverslips

SATURATED KOH SOLUTION PREPARATION

Mix ~100g of KOH pellets in ~250ml of absolute ethanol in a 1L beaker. Cover the beaker with aluminum foil to avoid spills. Stir this mixture using a magnetic stirrer at ~500 rpm until the solution turns dark orange in color (it would take approximately ~4 hrs). This step can be done overnight to save time.

Mean while load the glass coverslips/slides into Teflon holders (home built and can hold the glass along its periphery). Alternatively, if cleaning only one or two glass coverslips, one can hold coverslips using tweezers while cleaning.



Teflon holder filled with glass cover slips.

CLEANING GLASS COVERSLIPS/SLIDES

- Place beaker containing KOH solution in sonicator.
- Place two other beakers containing DI water into sonicator as well.
- Fill the soinicator with water to a level slightly lower than that of KOH solution in beaker (i.e. make sure the beakers are not floating in water).
- Turn on the sonicator (do not turn on the temperature i.e. Glass coverslip cleaning is done at room temperature).
- Place the Teflon holder filled with glass coverslips in the saturated KOH solution for 3 min. Move the holder up and down for better exposure of the coverslips to the KOH solution.
- Rinse the coverslips heavily with deionized water (18.2 MΩ*cm) using a squirt bottle
- Transfer the Teflon holder with coverslips into 2nd beaker to be sonicated in distilled deionized water for an additional 3 min.
- Remove the Teflon holder with coverslips from beaker and rinse heavily with distilled deionized water using a squirt bottle to make sure all the KOH solution is removed from the glass coverslips.
- Transfer the Teflon holder containing glass coverslips into final beaker to be sonicated in distilled deionized water again for another 3 min.
- At the end, remove the Teflon holder with coverslips from beaker and rinse the coverslips with 95% ethanol, and dry using ultra high purity nitrogen or argon gas.
- Coverslips should be stored in desiccator.

PLASMA CLEANING PRIOR TO USE

Before use, glass surfaces should be plasma cleaned (Harrick Plasma PDC-001) in oxygen for ~10 min at ~250 mTorr using ~30 W forward RF power. This will render them hydrophilic.

REFERENCE

Nagaraju Chada, Krishna P. Sigdel, Raghavendar Reddy Sanganna Gari, Tina R. Matin, Linda L. Randall, and Gavin M. King. '***Glass is a viable substrate for precision force microscopy of membrane proteins***', *Scientific Reports* **5,** 12550 (2015). https://doi.org/10.1038/srep12550