

# **Dogic Lab – Acrylamide Coating Protocol**

Updated January, 2016 by Stephen J. DeCamp.

This protocol is for coating two sets of containers of microscope glass slides and cover slips (approximately 20 pieces of glass). It requires roughly 300ml to fill the two sets of containers.

It is important to use DI water at all steps to prevent contaminants from dirtying the glass. Keep glass containers covered at all times to prevent dust from falling into the solution.

## **Glass cleaning Protocol:**

### **Washing Slides**

1. Put slides and coverslips in respective containers
2. Fill containers with DI water
3. Add ~300ul Hellmanex to coverslip container and 500ul to glass slide container
4. Microwave coverslips until hot, but not boiling. The heat helps to disperse the soap.
  - a. You can also use hot DI water from the start if you don't have a microwave.
5. Sonicate containers for 10 minutes.
6. Rinse 3-5 times with DI water. Make sure all residual soap is rinsed off of slides.
7. Store slides immersed in DI water until needed for experiments or additional surface treatments.

### **Optional Surface test.**

8. Check surfaces of a couple slides and slips to see if any water beads up. If it does, there are unfavorable contacts and slides/slips must be washed again. Clean slides should be hydrophilic.

### **Additional Wash – Extra Clean slides for Acrylamide Coating**

1. Wash in detergent as above
2. Fill containers with ethanol and sonicate 5-10 minutes
3. Rinse 3-5 times with DI water.
4. Fill containers with NaOH (0.1M) and sonicate 5-10 minutes to etch surface
5. Rinse 3-5 times and store in DI water until needed for experiments or additional surface treatments.

## **Acrylamide surface coating**

This provides a sterically repulsive brush which prevents proteins from sticking to glass surfaces. It also provides a hydrophilic surface. **Safety Note:** Handle Acrylamide (particularly in dry form) with extreme care. It is a known neurotoxin and carcinogen. Refer to the lab safety manual for MSDS info.

Ahead of time, prepare an acrylamide stock solution:

Create 20% acrylamide stock under vent hood. Acrylamide is most dangerous while in powder form and when unpolymerized. When in liquid, nitril gloves need to be used. Alternatively, we have recently been purchasing 40% Acrylamide solutions pre-prepared as this is much safer than dealing with powder.

**ProTip:** Make the 2% Acrylamide solution and start degassing it before preparing the silane solution so that there is no down-time between the two steps.

### **Silane coating for acrylamide polymerization**

1. For two sets of slide and slip containers, make ~300ml of solution.
2. Prepare Silane-coupling solution
  - \*Solution unstable, prepare right before use.
  - 98.5% Ethanol (233g or ~300ml)
  - 1% Acetic Acid (3ml)
  - 0.5% Silane Agent (1.5ml) 3-(Trimethoxysilyl)propylmethacrylate
3. Pour silane-coupling solution into glass slide and slip containers immediately after it is prepared. Let it sit for 10-15 minutes.
4. Rinse 3-5 times with DI water.
5. Proceed to polymerize Acrylamide off of silane coated glass.

#### **Optional Check:**

6. Silane agent sometimes forms islands/clusters on surface that are visible with dark field. If this is a problem, immerse in methanol and sonicate for 5 minutes. Dispose of methanol in appropriate waste container.

### **Acrylamide polymerization off 3-(Trimethoxysilyl)propylmethacrylate-coated glass**

1. Make ~300ml of a 2% Acrylamide solution, from the Acrylamide stock.
  - a. 20% stock: 30ml of Acrylamide in 270ml of water.
  - b. 40% stock: 15ml of Acrylamide in 285ml of water.
2. Pump solution under vacuum for ~30 minutes to degas.
3. With Acrylamide solution on a stir plate:
  - a. Add 105ul TEMED (Tetramethylethylenediamine) to acrylamide solution
  - b. Then add 210mg of APS (Ammonium Persulfate) into solution.
4. Immediately pour solution over silane slides/coverslips after it is mixed
5. Wait at least a few hours for polymerization, usually use next day. Keep slides/slips immersed in the polymerized acrylamide solution until ready to use.
6. **Use within a few weeks.**
7. When ready to use, remove a single slide or slip from the container, rinse with DI water, and air dry.