PREPARATION OF COATED SLIDES

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Having used gelatin, chrome-gel, and polylysine protocols (among others) to prepare coated slides, the best way (in my humble opinion) is APTS. As follows is our lab protocol based on Angerer:

Protocol 05.01.02 - last updated 9/5/95

Reference: In situ hybridization to cellular mRNAs using radioactively labeled RNA probes. L. Angerer (1989) In: In situ hybridization: methods for detecting DNA and RNA sequences at cellular and subcellular resolution. Am. Soc. Cell Biol. Workshop Manual. pp 1-21. Based on the more complex technique of Gotlieb and Glaser (1976) BBRC 63:815-821.

HAZARDOUS CHEMICALS: Do not use this protocol if you are not aware of all the safety precautions required to work with these chemicals. Consult all material safety data sheets. Work in a fume hood with proper safety technique. Wear appropriate safety gear.

SUPPLIES: 3 Coplin jars; 1 stock bottle; forceps. They need to be clean and lint free. Wear gloves when making the solution and dipping the slides.

1) Make a 2% 3-aminopropyltriethoxysilane(Sigma) working solution. To 98 mL of acetone add 2 mL of 3-aminopropyltriethoxysilane. Pipet up and down a few times to mix the solution. Use a dedicated bottle for this solution.

2) Fill the first Coplin jar with enough stock solution to cover the slide. Put the lid on unused stock. As the 3-aminopropyltriethoxysilane evaporates, refill Coplin jar with fresh stock. Fill a second Coplin jar with approximately 50 mls of acetone. Fill a third Coplin jar with approximately 50 mls of dH2O.

3) Line the work space in the fume hood with paper towels and place about three or four test tube racks on top of them as rests to lean the slides against after dipping.

4) A pair of forceps is needed for dipping the slides. First, place between five and seven slides in the first jar (the acetone/aminopropyltriethoxysilane working solution). Wait about one minute before removing them.

5) Using the forceps, transfer these slides to the second Coplin jar (acetone). While these slides are sitting in the acetone for a minute, begin putting new slides into the first jar. If one is preparing large batches of slides, replace the acetone rinse periodically.

6) Transfer the slides in the second jar to the third jar (distilled water). Again, let the slides sit in the jar for about a minute. While waiting for these slides, continue to add slides to the first and second jars. If one is preparing large batches of slides, replace the distilled water periodically.

7) Before removing the slides from the distilled water, dip them up and down a few times to make sure they have been rinsed well. Using the forceps, lean the slides against the test tube racks in order for them to dry.

8) Repeat this process until sufficient slides have been coated. When the slides are dry (about 10-15 minutes), store them in a labeled slide box. The slides should be clear. White spots are a sign of improper rinsing.

9) When finished dipping the slides, clean up the hood. Do not save the stock solution longer than a couple of hours. Place the stock solution from the stock bottle and first Coplin jar into the appropriate hazardous waste bottle. Rinse these two containers with the acetone from the second Coplin jar. Discard the acetone into the appropriate hazardous waste bottle. The distilled water may be disposed of in the sink. The three Coplin jars and stock jar should be placed neatly off to the side within the hood to await future use. ■

It is rather remarkable how slow American chemists have been in realizing the importance of the microscope as an adjunct to every chemical laboratory . . . (The microscope is) as much a necessity in every analytical laboratory as is the balance.

E.M. Chamot, J. Appl. Microscopy 2:502 (1899)

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Dr. R. Bruch
Louisiana State University,
Department of Biological Sciences
508 Life Sciences Building
Baton Rouge, LA 70803

Or submit by email: rbruch@lsu.edu

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