

Coating standard glass microscope slides with poly-l-lysine

Overview

The outlined protocol was based on the method described by Pat Brown (<http://cmgm.stanford.edu/pbrown/>).

Equipment and reagents

- Horwell Premium plain microscope slides: 76 x 26 x 1.0 - 1.2 mm (Scientific Laboratory Supplies; Cat. No. MIC3008)
- Poly-L-Lysine solution (Sigma; Cat. No. P8920)
- Polyacetal staining troughs (BDH; Cat. No. 406/0236/00)
- Glass staining troughs (BDH; Cat. No. 406/0230/10)
- Microscope slide box (for centrifugation) (BDH; Cat. No. 406/0286/00)
- Savant DNA 120 SpeedVac
- MilliQ water
- Graduated glassware for cleaning solution
- Graduated plastic ware for Poly-L-Lysine solution
- Magnetic Stirrer Unit & Stirrer Bar or Orbital Shaker

Procedure

1. Place the microscope slides into Polyacetal staining racks. Discard slides that are damaged, misshapen or too dirty.
2. Prepare the cleaning solution: (Gives enough volume for one glass-staining trough). Dissolve 35 g NaOH in 140 ml MilliQ water, using a magnetic stirrer until completely mixed, then add 210 ml of 96% ethanol, and mix completely. If the solution is not clear add more MilliQ water.
3. Place the Polyacetal staining rack into the glass staining trough and pour the cleaning solution over the slides, making sure they are completely covered with the solution. Then place a lid on the glass staining trough to stop evaporation.
4. Place stirrer bar in trough and mix on magnetic stirrer or Orbital Shaker for 2 hours.
5. Rinse slides in MilliQ water at least four times (for 1 minute) by plunging the slides up and down in polyacetal staining troughs containing the MilliQ water (using fresh MilliQ water each time). After rinsing keep stored under fresh MilliQ water until ready for step 7.
6. Prepare fresh Poly-L-Lysine slide coating solution: Add 25 ml of Poly-L-Lysine solution to 225 ml MilliQ water (Enough for one polyacetal staining trough). Do not use a glass-staining trough for the Poly-L-Lysine coating step.
7. Cover slides with the fresh Poly-L-Lysine slide coating solution and leave for between 5 to 10 minutes with gentle agitation on an orbital shaker (50 rpm).
8. Transfer slides into slide centrifuge boxes making sure to leave space at the ends for any liquid collected and speedvac for 5 minutes on medium heat (43 °C) using a Savant DNA 120 Speedvac with a microtitre plate rotor (1725 rpm). It is crucial that some space is left at the ends of the centrifuge boxes otherwise any solution that builds up against the end slides during centrifugation will cause uneven coating. The centrifugation step can be done in batches, leaving slides in solution until they can be centrifuged.
9. Store the slides in a microscope slide box in a cool, dry and dust-free cupboard.

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