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DABCO Mounting Slide Protocol for *Drosophila melanogaster* embryos

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Protocol status: Working
We use this protocol and it's working

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Keywords: mounting drosophila melanogaster embryo, drosophila melanogaster embryos protocol, dabco mounting slide protocol, drosophila melanogaster embryo, pbt onto slide, confocal microscopy, slide, conventional fluorescence, fluorescence

Abstract

Protocol for mounting Drosophila melanogaster embryos stored in PBS or PBT onto slides for conventional fluorescence and/or confocal microscopy.

Adapted from:

Kosman, D., Mizutani, C.M., Lemons, D., Cox, W.G., McGinnis, E., and Bier, E. (2004)
https://science.sciencemag.org/content/305/5685/846.long

Materials

MATERIALS

- 1X PBS (Phosphate-buffered saline)
- Microscope slides
- Glycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G5516
- 14-Diazabicyclo[2.2.2]octane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D27802
- clear nail polish

Safety warnings

Read SDS for DABCO prior to starting this protocol!

Make Mountant

1 In light-shielded 50 mL conical (I cover mine in foil) add 1.25 g of DABCO crystals, 15 mL of 1X PBS, and 35 mL of glycerol.

Safety information

Read SDS for DABCO before starting.

2 Mix on rocking platform until the solution is homogeneous. Store at -20 °C .

Mount Slides

3 Take off as much liquid as you can, resuspend embryos in an arbitrary amount of DABCO mountant (depends on how dilute you want the embryos to be, but generally a couple hundred ul). Allow the embryos to settle 01:00:00 to 03:00:00 at Room temperature or overnight at 4 °C before mounting. Embryos can be stored for months/years in microcentrifuge tubes in light-shielded boxes at -20 °C .

4 Transfer approximately 35 µL of embryos with using pipette tip cut with scissors onto a clean slide between two 22 × 22 mm coverslips approximately 0.5 cm apart in the middle of the slide. Cover with another 22 × 22 mm cover slip. Seal the edges with a quick-drying, transparent nail polish. Slides can be stored for a few weeks at 4 °C in the dark (or a few months at -20 °C) .

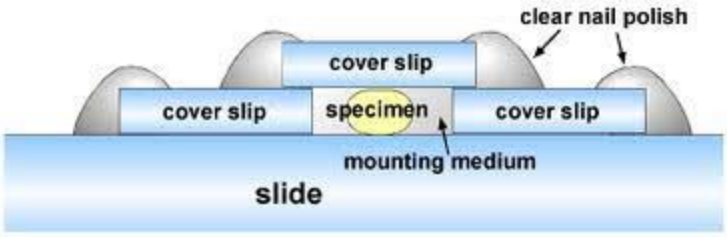

Image source:
https://www.uwo.ca/sci/research/biotron/pdf/microscopy/LSM_SampleGuide.pdf

Note

Keep in mind if you're DAPI staining, that tends to diffuse away. It is better to mount a fresh aliquot of embryos if samples are reanalyzed at a later date.

5 Analyze embryos by conventional fluorescence or confocal microscopy.