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DABCO Mounting Slide Protocol for *Drosophila melanogaster* embryos

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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

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1X PBS (Phosphate-buffered saline) Contributed by users

Microscope slides Contributed by users

Glycerol Sigma Aldrich Catalog #G5516

14-Diazabicyclo[2.2.2]octane Sigma-aldrich Catalog #D27802

clear nail polish Contributed by users

SAFETY WARNINGS

Read SDS for DABCO prior to starting this protocol!

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.2yjgfun

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<https://dx.doi.org/10.17504/protocols.io.2yjgfun>

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

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PROTOCOL integer ID:
 23275

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 x 22 mm coverslips approximately 0.5 cm apart in the middle of the slide. Cover with another 22 x 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.