DABCO Mounting Slide Protocol for *Drosophila melanogaster* embryos

Ashley Albright[1]

[1]University of California, Berkeley

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

May 17, 2019

Working

Ashley Albright

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

NAME	CATALOG #	VENDOR
1X PBS (Phosphate-buffered saline)		
Microscope slides		
Glycerol	G5516	Sigma Aldrich
14-Diazabicyclo[2.2.2]octane	D27802	Sigma-aldrich
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.

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) .

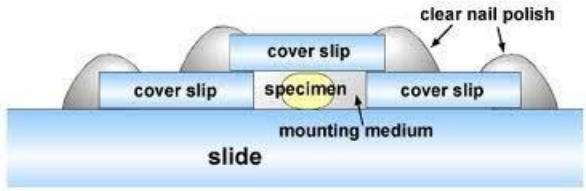

Image source: https://www.uwo.ca/sci/research/biotron/pdf/microscopy/LSM_SampleGuide.pdf

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.