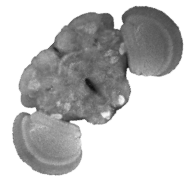


## Glycerol Mounting

- All tissues are protected from light
- All tissue and solutions, including SlowFade Gold are at room temperature (RT).
- For a video demonstrating glycerol mounting see Glycerol Mounting of Adult CNS.

1. **Prepare your slide.**
  - Peel and stick the multi-well adhesive spacer to the slide. Burnish the top of the spacer to firmly adhere it to the slide. Use a scalpel to trim off any excess adhesive sticker overhanging the edges of the slide.
2. **Prepare dish.** Start with a clean 9-well glass dish. Run distilled water over a clean dish for a few minutes. Dry the dish with a Kimwipe and protect it from dust.
3. **Rinse.** Remove the phosphate buffered saline with 5% Triton X-100 (PBT) from each tube of tissue to be mounted, being very careful not to aspirate the tissue. Remove all but about 100  $\mu$ L. Fill each tube with PBS (~1.75 mL). Nutate the tubes for 20 minutes while protecting from light.
  - Rinsing with PBS removes the Triton in PBT. Any lingering Triton will prevent the tissue from adhering to the slide during mounting. If the tissues don't stick to the slide, they will move when adding mounting medium and when the coverslip is placed.
4. **Arrange tubes.** When nutation is finished, place the tubes upright in test tube rack and allow the brains to settle to the bottom (~2 minutes). Arrange the tubes in the rack according to your mounting plan.
5. **Transfer tissue to glass wells.** Add 1 mL of PBS to the glass dish. Transfer the contents of a tube to the glass dish with a pipette. Add a small amount of PBS to the tube to suspend any tissue that was left behind. Transfer this small amount of PBS and tissue, if present, to the glass dish.
6. **Add 2:1 SlowFade to the slide.** Add 9  $\mu$ L of 2:1 SlowFade Gold to each well of your slide. Spread the SlowFade around within each well using the pipet tip.
  - Mix the 2:1 solution before adding by aspirating and expelling the solution several times within the tube using a pipette.
7. **Transfer tissue to the slide.**
  - Using your slide map as your guide, transfer the brains to the appropriate well with 2:1 SlowFade Gold using a pipette.
8. **Remove the 2:1 SlowFade.** Aspirate most of the 2:1 solution being very careful not to remove the tissue. Leave just enough solution to allow the tissue to be moved easily.
9. **Arrange the tissue.**
  - Wet your brush with clean PBS before use. Clean your brush frequently by dipping it in fresh PBS.
  - Use your mounting brush to remove any noticeable debris from the well.
  - Using the brush, orient the brains anterior up. Position them in their approximate final location at a 45° angle with the ventral edge facing the bottom right corner of the slide.
10. **Remove debris.** Aspirate the remaining 2:1 SlowFade Gold and replace it with fresh 2:1. Repeat this as needed to remove any debris. Debris will interfere with imaging. Reposition the brains if they have moved.



**11. Adhere tissue to the slide.**

- Aspirate as much 2:1 SlowFade Gold as possible. Reposition the brains as needed.
- *Let the brains sit for 3 minutes.* This waiting period allows the brains to adhere to the slide preventing them from moving. The residual SlowFade coating the tissue will prevent them from drying out.

**12. Add 100% SlowFade.** Add 9  $\mu$ L of 100% SlowFade to the well. If the tissue does not move, proceed to the next step. If the tissue moves:

- remove the 100% SlowFade
- add 2  $\mu$ L of PBS and reposition the tissue
- remove the solution around the tissue and wait 3 minutes for the tissue to adhere
- after 3 minutes, add 9  $\mu$ L 100% SlowFade

**13. Repeat** steps 7-13 until all the tubes have been mounted.**14. Add cover glass.**

- Clean the cover glass with lens paper to remove dust and debris.
- Place the cleaned cover glass onto the slide.
- Use a Thirsty Stix or twisted tip of a Kimwipe to absorb excess SlowFade that oozes out from under the cover glass.

**15. Inspect the slide for bubbles** while viewing with the dissecting microscope. If bubbles are touching the tissue, slowly slide the cover glass with forceps until the bubble has moved away from the tissue.

- Do not lift the cover glass. This will damage the tissue or result in tissue loss.

**16. Seal with nail polish.** Add small dabs of nail polish to several points on the perimeter of the cover slip. When dry (~5 minutes), brush nail polish along the entire perimeter of the cover glass. Let the nail polish dry thoroughly.

- When dry, the initial dabs of nail polish hold the cover glass in place so that it doesn't move when brushing nail polish along the perimeter of the cover glass. If the cover glass is not held in place, it will move when adding nail polish to the perimeter. This may prevent the nail polish from sealing the cover glass to the slide.

## How to Prepare SlowFade Gold Aliquots

**100% SlowFade aliquot**

1. Add 1.5 mL of SlowFade Gold to a 1.5 mL amber tube.
2. Store at 4°C.

**2:1 SlowFade aliquot**

1. Add 1000  $\mu$ L SlowFade to a 1.5 mL amber tube.
2. Add 500  $\mu$ L PBS to the SlowFade.
3. To mix the 2:1 solution, use a P-20 to aspirate and expel the solution in the amber tube several times. Avoid introducing air bubbles.
4. Store at 4°C.

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### Long-term storage of mounting media stock

- SlowFade Gold – store at -20°C

### Long-term storage of slides

- Store prepared slides flat in slide books at 4°C

### Reagents and Supplies

- 9-well Pyrex plate (glass dish). Electron Microscopy Sciences. # 71563-01
- Clear Nail Polish. Electron Microscopy Sciences. # 72180
- Cover glass: square, No. 1, 22 mm. Fisher Scientific. # 12-542 B
- Microcentrifuge tubes, amber, 1.5 mL. Eppendorf. # 022363221
- PBS - Phosphate Buffered Saline, 1X. Cellgro. # 21-040
- Red Sable brush. Electron Microscopy Sciences. # 66100-50
- SecureSeal Imaging Spacers, Grace Bio-Labs, 8 wells. Contact company for ordering information.
- SlowFade Gold AntiFade Mountant. Life Technologies. # S36936
- Superfrost Plus Slides, 25x75 mm. Fisher Scientific. # 12-550-15
- Thirsty Stix. Electron Microscopy Sciences. # 70996