Miowol Recipe (Antifade reagent)

1. Put 6 g glycerol in a 50 ml plastic centrifuge tube and add a small stir bar. (note--Grams not ml)
2. Add 2.4 g Miowol (Calbiochem); stir to mix.
3. While stirring, add 6 ml distilled water and leave 2 hours at room temperature.
4. Add 12 ml 0.2M Tris (pH 8.5).
5. Add a small amount of NaN3 (Sodium Azide) such that the final concentration is .02% (optional--I do not do this normally).
6. Incubate the tube in hot water (50 – 60ºC) for 10 minutes to dissolve the Miowol. This can be repeated over several hours if necessary.
7. Add Dabco (Sigma D27802) for a final 2.5% (w/v) and vortex until dissolved.
8. Centrifuge at 5000 g for 15 minutes to remove any undissolved solids. Store 1ml aliquots in Eppendorf tubes at -20ºC.
9. Warm tubes to room temperature for use. Opened tubes can be stored at 4ºC for approximately 1 month. Discard if any crystalline material is seen in the tube or on the slides.
10. Leave coverslipped slides in the dark overnight to harden before oil immersion lenses are used.
11. Store slides in -20.

Note: Do not use so much mounting solution that the coverslips are floating. Normally, 15 – 20 ul is sufficient for a 22 x 22 mm coverslip. 22 x 50 mm coverslips require about 40 – 50 ul.