PVA-DABCO Coverslipping Solution for Immunofluorescence

This solution is a glycerol based mounting medium containing an anti-fading reagent for use with immunofluorescence preparations. The same solution may be used with or without DABCO for "aqueous" mounts of material for brightfield microscopy. The advantage of this solution is that the medium at the perimeter of the coverslip polymerizes upon contact with air forming a strong seal. There is no need to seal with nail polish. The coverslips can be removed by soaking the slide in buffer.

Mount stained sections on glass slides and let briefly air dry (less than one minute) or if using Lab-Tek chamber slides, remove the chamber and tip to remove excess buffer. Apply 75 µl of PVA-DABCO solution for use with a 50 mm coverslip. Use caution with pipetting as air bubbles are easily introduced if the solution is aspirated in any way. I suggest using a repeating pipetteman.

Lay the coverslipped slides flat overnight until polymerization is complete. Afterwards, the coverslips may be cleaned with solvents to remove immersion oil and slides can be stored upright in normal slide storage boxes. Store slides in slide boxes at 4°C or -20°C and preparations should remain usable for at least five years, the longest time point evaluated to date.

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For 25 ml of 2.5% PVA-DABCO
Make 25 ml of PVA-DABCO in 50 ml culture tube to allow ample room for mixing.

1. Using a 50 ml culture tube, weigh 6 gm of glycerol into the culture tube on the balance
2. Add 2.4 gm of PVA
3. Mix well by repeatedly inverting the tube until the PVA is coated with glycerol
4. Add 6 ml of distilled water and repeat mixing until relatively uniform
5. Mix overnight using a rotator at room temperature
6. Add 12 ml of 0.2 M Tris-HCl at pH8-8.5
7. Heat to 50°C in a water bath with mixing (approximately 30 min)
8. Add 0.625 gm of DABCO and mix well
9. Centrifuge at 5000g for 15 min
10. Remove supernatant, aliquot (suggest 1 ml), and store at -20°C

The solution may be kept up to 6 months at -20°C and up to one week at 4°C before becoming milky. Do not refreeze.

Stock materials	*Vendor*	*Location*
PVA-polyvinyl alcohol	Sigma (P8136)	Chemical Shelf
DABCO- 1,4 diazabicyclo [2.2.2]octane	Sigma (D2522)	Chemical Fridge
Tris-HCl	Sigma (T3253)	Chemical Shelf
Glycerol	Fisher G33-1	Chemical Shelf

To make 0.2N Tris HCl:

500 ml ddH_2O
15.76g Tris HCl
add7 pellets NaOH
adjust pH to 8.0-8.5