

DABCO Mounting Medium Recipes

Adapted from: Johnson, GD, Davidson, RS, McNamee, KC, Russell, G, Goodwin, D, and Holborow, EJ, Fading of Immunofluorescence during microscopy: a Study of the Phenomenon and its Remedy, *Journal of Immunological Methods*, 55 (1982)231-242.

Materials:

1,4-Diazobicyclo-(2,2,2)octane (Sigma #D2522)
Glycerol, spectrophotometric grade (Kodak)
1XPBS
HCl (diluted 1:20 with H₂O)

Methods:

Mix 25 mg/ml of DABCO in 90% glycerol/10% 1xPBS.
You may gently heat and or rock the mixture to dissolve.
Adjust pH to 8.6 with diluted HCl.

For example, to make 25 ml final volume:

625 mg Sigma DABCO
22.5 ml glycerol
2.5 ml 1xPBS
Adjust pH to pH 8.6 with diluted HCl.

Indig et al., *Biochem J.* 327:291-8, 1997

Dissolve in beaker (0.5 L for 100 mL solution):

- DABCO (1,4-Diazabicyclo[2.2.2]-octane, Aldrich) 2.5% (w/v)
- Mowiol 4-88 (Calbiochem) 10% (w/v)
- Dissolve in 0.1M TRIS-HCl, pH 8.5 and 25% (w/v) glycerol

This could take a few hours - use a heated magnetic stirrer at low speed, about 50 C.

When dissolved, aliquot 0.25 mL into microtubes, store at -20 C in the dark (good for well over one year).

Before use, heat to 65 C for 10 min, let cool 1-2 min and use immediately on specimen. Discard remains. This mounting media will solidify overnight in the refrigerator (4 C, dark), but is not as hard as epoxy mounts, so handle gently.

I measured the refractive index as $R=1.4101$ @ 22.0 C (water 1.33, oil 1.56)

From the Confocal Listserv: The people on the list judged these recipes favorably compared with commercial reagents like VectaShield and Molecular Probes "Slo-Fade" anti-fades, but there may be exceptions. Molecular Probes now has several formulations on the market. But Molecular Probes also has some info showing that even their own anti-fades diminished the photostability (over PBS) when used with the AlexaFluor dyes. Some anti fades from MP give a slower decay, but at the expense of lower initial fluorescence.

Anti-fade reagents to reduce photobleaching rates. These reagents are available from Sigma, and others.

1% DABCO in 90% glycerol 10% PBS (neutral pH).
if using FITC, adjust the pH to 8.0 - 8.5, as FITC is noticeably brighter at a higher pH.

4% n-propyl gallate in 90% glycerol 10% PBS (neutral pH)
(1-2 hr to dissolve; filter through coarse paper; store in brown bottle at RT)
(Giloh and Sedat; Science, 1982)