Antifade

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Antifade: 1,4-Phenylenediamine

(This is the preferred antifade reagent)

Reagents

- 1,4-Phenylenediamine dihydrochloride (Sigma, 78460)
- Glycerol (Invitrogen, 15514-011)
- Phosphate Buffered Saline (PBS), 1X (Gibco, 10010-023)
- Sodium bicarbonate (Mallinckrodt, 7412)
- Sodium carbonate (Sigma, S7795)

Preparation

Carbonate-Bicarbonate Buffer (pH 9.0) - 5ml:
Sodium bicarbonate, 0.4M
Sodium carbonate, 0.1M
H_2O, 5ml
If needed adjust pH to 9

Procedure

1. Prepare carbonate-bicarbonate buffer (pH 9.0).

2. Dissolve 50 mg 1,4-phenylenediamine in 2 ml of PBS.

3. Adjust pH with carbonate-bicarbonate buffer to 8.0.

Important: Add 2 ml buffer and check pH, then add dropwise until pH is 7.99-8.00. If pH exceeds 8.0, the procedure must be started over.

4. Complete volume to 5 ml with PBS and transfer to a 50 ml tube.

5. Add 38.7ml glycerol 100% and 6.3ml H_2O. Leave on inverter for at least 1 hr.

6. Aliquot in opaque tubes and store at -20°C.
Note: After storage, if the antifade is still clear it should be good, but if it looks brown in the tube or orange under microscope it needs to be changed.

Antifade 2: DABCO (1,4 diazabizyclo[2.2.2]octane)
(alternative)

Reagents

- DABCO (1,4-diazabicyclo[2.2.2]octane) (Sigma, Cat. D2522)
- Glycerol (Invitrogen, 15514-011)
- Tris-HCl, l M pH 8.0
- Sterile H_2O

Preparation

Components Amount:
DABCO, 0.233 g
Tris-HCl (pH 8.0) 1 M, 200 µl
Sterile H_2O, 2.06 ml
Glycerol, 7.74 ml

Procedure

1. Combine components.

2. Dissolve by warming to 70°C.

3. Vortex.

4. Aliquot and store at -20°C.