Antifade

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

Antifade 1: 1,4-phenylene-diamine
(This is the preferred antifade reagent)

Reagents

1,4-phenylene-diamine
Sigma, Cat. P1519, 100 g
Glycerol, 86%
Phosphate Buffered Saline (PBS), 1X
Sodium bicarbonate
Sodium carbonate

Preparation

Carbonate-Bicarbonate Buffer (pH 9.0)

Sodium bicarbonate, 0.5 M (pH 8.13)	4 ml
Sodium carbonate, 0.5 M (pH 11.32)	1 ml
Filter sterilize	

Procedure

1. Prepare carbonate-bicarbonate buffer (pH 9.0).
2. Dissolve 50 mg 1,4-phenylenediamine in 2 ml 1X PBS.
3. Adjust pH with carbonate-bicarbonate buffer to 8.0.
4. Add 1X PBS to 5 ml. **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.
5. Mix with 45 ml 86% glycerol. Leave on inverter for at least 1 hr.
6. Aliquot and store at -20°C.

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

Reagents

DABCO (1,4-diazabicyclo[2.2.2]octane)
Sigma, Cat. D2522
Glycerol, 86%
Tris-HCl, l M pH 8.0
Water, sterile

Preparation

Components	Amount
DABCO	0.233 g
Tris-HCl, 1 M pH 8.0	200 µl
Sterile water	800 µl
Glycerin, 86%	9 ml

Procedure

1. Combine components.

2. Dissolve by warming to 70°C.

3. Vortex.

4. Aliquot and store at -20°C.