PCA/PCD/Trolox Antifade for IF

100mM Trolox Stock Solution

Trolox: (t)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 250.29 g/mol

1. Sonicate 1G Trolox in 3.99ml methanol
2. Add 35.96ml DI water
3. pH to 9.5 with 1M NaOH

-make 300ul aliquots

100mM PCA Stock Solution

PCA: 3,4-Dihydroxybenzoic acid 154.12 g/mol

1. Dissolve 50mg PCA in 3.244ml DI water (sonicate if necessary)
2. pH to 9.0 using 1M NaOH

-make 300ul aliquots

PCD Stock Solution **Store at -20C**

PCD: Protocatechuate 3,4-dioxygenase 700,000 g/mol

Very small volumes. PCD units contain 40% stabilizer.

1. Prepare 2mM EDTA
2. Prepare 200mM Tris-HCL pH 8
3. Add 8.3mg glycerol to PCD vial and sonicate repeatedly until dissolved
4. Add 4.15ul of 2mM EDTA
5. Add 4.15ul of 200mM Tris-HCL pH8
6. Add 0.062G KCl
7. Sonicate repeatedly until dissolved

-make 1ul aliquots

Working Solution

Email thread suggestion: Add 10ul Trolox, 20ul PCA, and 1 ul PCD into each 1ml of DI water.

Suggested concentrations from paper: 1mM Trolox, 2.5 mM PCA, 10nM PCD