Schwer Lab

5× Direct-loading PCR buffer

Optimized by Annika Carlson

REAGENTS

1 M KCl (MW =74.55 g/mol)
Dissolve 3.728 g KCl in 30 mL in 50-mL conical tube. Mix until dissolved. Bring volume to 50 mL with ddH_2O. Store at RT.

1 M Tris-Cl, pH 9.0 (MW=121.14 g/mol)
Dissolve 6.057 g Tris base into 40 mL ddH_2O. Adjust the pH to 9.0 by adding concentrated HCl (~2.1-3.0 mL) while stirring at RT. Bring the volume to 50 mL by adding ddH_2O. Store at RT.

10% (w/v) IGEPAL CA-630 in ddH_2O

1 M $(NH_4)_2SO_4$ (MW = 132.14 g/mol)
Dissolve 6.607 g $(NH_4)_2SO_4$ in 35 mL ddH_2O. Bring the volume to 50 mL by adding ddH_2O. Store at RT.

1 M $MgCl_2 \bullet 6H_2O$ (MW = 203.30 g/mol)
Dissolve 10.165 g $MgCl_2 \bullet 6H_2O$ in 35 mL ddH_2O. Bring the volume to 50 mL by adding ddH_2O. Store at RT.

Cresol red ($C_{21}H_{18}O_5S$; Sigma 114472-5G; MW = 382.43 g/mol)
Migrates at about 50-bp DNA fragment size.

Sucrose: S0389-500G (Sigma)

PROCEDURE

5× Direct-loading PCR buffer [30% (w/v) sucrose, 15 mM $MgCl_2$, 50 mM KCl, 40 mM $(NH_4)_2SO_4$, 0.03% (w/v) Cresol red, 0.25% (w/v) IGEPAL CA-630, 50 mM Tris-Cl, pH 9.0]

Dissolve 3 g sucrose in 5 mL ddH_2O
add 150 µL 1 M $MgCl_2 \bullet 6H_2O$
add 500 µL 1 M KCl
add 400 µL 1 M $(NH_4)_2SO_4$
add 500 µL 1 M Tris-Cl, pH 9.0
add 0.003 g Cresol red
add 250 µL 10% IGEPAL CA-630

Mix well. Centrifuge briefly. Bring total volume to 10 mL with ddH_2O. Sterilize with 0.22 µm filter. Prepare 1-mL aliquots and store at -20˚C.