

Solutions and buffers

0.1 M CaCl₂

1.47 g of Calcium Chloride (CaCl₂.2H₂O) was dissolved in 100 ml of MQ water and autoclaved at 15 lb/sq. in for 15 min and stored at 4°C.

0.1 M CaCl₂ with 20 % glycerol

1.47 g of calcium chloride (CaCl₂.2H₂O) was dissolved in 60 ml of MQ water and 20 ml of glycerol was added. Solution was autoclaved at 15 lb/sq. in for 20 min and store at 4°C.

STET buffer

8 g of sucrose, 0.1 g TritonX-100, 50 mM Tris (pH 8.0) and 50 mM EDTA (pH 8.0) were dissolved in 80 ml of distilled water and final volume was made up to 100 ml. Solution was autoclaved at 15 lb/sq. in for 20 min and store at room temperature.

1M Sorbitol

182 g sorbitol (Himedia, India) was dissolved in 800ml of H₂O, and final volume was adjusted to 1L, autoclaved at 121°C for 15 minutes at 15 psi.

STC Buffer

50 mM Tris and 50 mM CaCl₂ were added to 1M sorbitol solution and autoclaved at 121°C for 15 minutes at 15 psi.

PTC

400 g of PEG 3350 (Sigma, USA) was added to 600 ml of PTC buffer and volume was made up to 1 liter. The solution was autoclaved at 121°C for 15 minutes at 15 psi.

CMS (complete medium sorbitol)

182 g Sorbitol (Himedia, India) was dissolved in 800ml of complete medium and final volume was adjusted to 1L with complete medium, autoclaved at 121°C for 15 minutes at 15 psi.

Molten regeneration medium

0.4 g of agarose (Invitrogen, USA) was added in 100 ml complete medium and autoclaved at 121°C for 15 minutes at 15 psi.

DEPC-treated water is made by dissolving diethylpyrocarbonate (DEPC) in distilled water to a final concentration of 0.1 %. It was then incubated at 37 °C for one hour and sterilized by autoclaving.

1X PBS (Phosphate Buffer Saline)

8 g of NaCl, 2 g of KCl, 1.44 g of Na₂HP04 and 0.2 g of KH₂P04 were dissolved in 800 ml of MQ water. pH was adjusted to 7.4 with HCl. Final volume was brought up to 1 L and sterilized by autoclaving at 15 lb/ sq.in for 20 min and stored at RT.

0.5 M EDTA

Dissolve 186.1 g of EDTA (disodium dihydrate salt) in 700 ml of MQ water. Keep on shaking vigorously. Adjust pH to 8.0 with 10 M NaOH solution. EDTA will not dissolve completely until the pH of the solution reaches 8.0. Finally, add water up to 1 L and autoclave at 15 lb/sq.in for 20 min. Store at RT.

1 M Tris (pH 6.8, 8.0, 8.8, 9.5)

Dissolve 121.1 g of Tris base in 800 ml of MQ water. Adjust the pH to a desired value by adding concentrated HCl. Make up the final volume to 1 L with MQ water and sterilize by autoclaving at 15 lb/sq.in for 20 min. Store at RT.

10 mg/ml EtBr

Dissolve 10 mg of EtBr in 1 ml of autoclaved MQ water. Store at RT in a dark bottle.

6x DNA loading dye

Dissolve Bromophenol blue (0.25% w/v); Xylene cyanol FF (0.25% w/v); Glycerol (30% v/v) in water. Store at 4°C.

20 x SSC

Dissolve 3 M Sodium citrate (175 g/l) and 0.3 M Trisodium citrate (88 g/l) in distilled water. Adjust pH to 7.0 with 1 N HCl and then autoclave at 15 lb/sq.in for 20 min. Store at RT.

Antibiotic solutions**Ampicillin**

Prepare 100 mg/ml stock solution in double distilled sterile water and store at 4°C. Final concentration used is 100 µg/ml.

Kanamycin

Prepare 50 mg/ml stock solution in double distilled sterile water and store at 4°C. Final concentration used is 50 µg/ml.

Streptomycin

Prepare 50 mg/ml stock solution in double distilled sterile water and store at 4°C. Final concentration used is 50 µg/ml.