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| **Tris-Cl (1 M)** Dissolve 121.1 g of Tris base in 800 ml of H2O. Adjust the pH to the desired value by adding concentrated HCl <!>. pH HCl 7.4 70 ml 7.6 60 ml 8.0 42 ml ßour most common Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 liter with H2O. Dispense into aliquots and sterilize by autoclaving. If the 1 M solution has a yellow color, discard it and obtain Tris of better quality. The pH of Tris solutions is temperature dependant, and decreases ~0.03 pH units for each 1 C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5 C, 25 C, and 37 C, respectively.  **20X SSC** Dissolve 525.9 g of NaCl and 264.6 g of sodium citrate in 2.4 liters of H2O. Adjust the pH to 7 with a few drops of a 14 N solution of HCl <!>. Adjust the volume to 3 liters with H2O. Dispense into aliquots. Sterilize by autoclaving. The final concentrations of the ingredients are 3.0 M NaCl and 0.3 sodium citrate.  **Extraction/Lysis Buffers and Solutions** Alkaline Lysis Solution I (Plasmid Preparation) 50 mM glucose 25 mM Tris-Cl (pH 8.0) 10mM EDTA (pH 8.0)  Prepare solution I from standard stocks in batches of ~500 ml, autoclave for 15 minutes at 15 psi (1.05 kg/cm2) on liquid cycle, and store at 4 C.  **Alkaline Lysis Solution II (Plasmid Preparation)** 5 M potassium acetate 180.0 ml glacial acetic acid <!> 34.5 ml H2O 85.5 ml  The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. Store the solution at 4 C and transfer it to an ice bucket just before use.  **Electrophoresis and Gel-loading Buffers** **TAE 50 X** (stock solution/Liter) 242 g of Tris base 57.1 ml of glacial acetic acid <!> 100 ml of 0.5 M EDTA (pH 8.0)  **TBE 5X (3.5 Liters)** 189 g of Tris base 96.25 g of boric acid 70 ml of 0.5 M EDTA (pH 8.0)  **LB Freezing Buffer** 36 mM K2HPO4 (anhydrous) 13.2 mM KH2PO4 1.7 mM sodium citrate 0.4 mM MgSO4\*7H2O 6.8 mM ammonium sulfate 4.4% (v/v) glycerol 100 ml LB broth  Dissolve the salts into 100ml of LB to the specified concentrations. Measure 95.6 ml of the resulting solution into a fresh container and then add the 4.4 ml of glycerol. Mix the solution well and then sterilize by passing it through a 0.45-ul disposable Nalgene filter. Store the sterile freezing medium at a controlled room temperature (15-25 C).  **EDTA (0.5 M, pH 8.0)** Add 186.1 g of disodium EDTA\*2H2O to 800 ml of H2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ~8.0 by the addition of NaOH.  **NaCl (Sodium Chloride, 5 M)** Dissolve 292 g of NaCl in 800 ml of H2O. Adjust the volume to 1 liter with H2O. Dispense into aliquots and sterilize by autoclaving. Store the NaCl solution at room temperature.  **SDS (20% w/v)** Also called sodium lauryl sulfate. Dissolve 200 g of electrophoresis –grade <!> in 900 ml of H2O. Heat to 68 C, and stir with a magnetic stirrer to assist dissolution. If necessary, adjust the pH to 7.2 by adding a few drops of concentrated HCl <!>. Adjust the volume to 1 liter with H2O. Store at room temperature. Sterilization is not necessary. Do not autoclave.  **Sodium Acetate (3 M, pH 5.2 and pH 7.0)** Dissolve 102.07 g of sodium acetate\*3H2O in 200 ml of H2O. Adjust the pH with glacial acetic acid <!> or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 250 ml with H2O. Dispense into aliquots and sterilize by autoclaving.  **5X Denaturing Solution**  140g NaOH 613.6g NaCl bring to 3.5L H2O autoclave  **2X Neutralizing Solution**  ~2L H2O 613.63g NaCl 363.42g Tris pH to 7.5 with concentrated HCl bring volume to 3.5L with H2O autoclave  **CTAB Extraction Buffer**   |  |  |  | | --- | --- | --- | | **Final Con.** | **Stock Conc** | **Amt of stock to add** | | 0.1 M Tris pH 7.5 | 1 M Tris pH 7.5 | 100ml | | 0.75M NaCl | 5 M NaCl | 140ml | | 0.01M EDTA | 0.5M EDTA | 20ml | | 1% CTAB | CTAB | 10g | | ddH2O |  | 730ml | |  |  | **1000ml** | | 1% B-mercaptoethanol | 14 M BME | 10 ml/1000 ml CTAB Buffer (add just before use) |   CTAB =1% mixed alkyl trimethyl-ammonium bromide  DNA Wash Solution 76% ethanol \_\_\_\_\_\_\_\_\_\_\_\_ 304 ml of 100% ethanol 10 mM NH4Ac \_\_\_\_\_\_\_\_\_ 0.4 ml of 10 M NH4Ac Bring volume to 400 ml with ddH2O.  **Antibiotics**   |  |  |  |  |  | | --- | --- | --- | --- | --- | |  | Stock Solution Concentration | Storage | Working Concentration Stringent Plasmids | Working Concentrations Relaxed Plasmids | | Ampicillin | 50 mg/ml in H2O | -20 C | 20 ug/ml | 20 ug/ml | | Carbenicillin | 50 mg/ml in H2O | -20 C | 20 ug/ml | 60 ug/ml | | Chloramphenicol | 34 mg/ml in ethanol | -20 C | 25 ug/ml | 170 ug/ml | | Kanamycin | 10 mg/ml in H2O | -20 C | 10 ug/ml | 50 ug/ml | | Streptomycin | 10 mg/ml in H2O | -20 C | 10 ug/ml | 50 ug/ml | | Tetracycline | 5 mg/ml in ethanol | -20 C | 10 ug/ml | 50 ug/ml | |  |  |  |  |  | |  |  |  |  |  | |

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| 0.25% (W/V) Bromophenol blue |
| 0.25% (W/V) Xylene cyanol FF |
| 30% (V/V) Glycerol in H2O store at 4C |