Commonly Used Buffers and Solutions

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BD-I Solution for Alkaline Lysis DNA Minipreps

50mM glucose ; 25mM Tris-Cl (pH 8.0) ; 10mM EDTA (pH 8.0)

For 500ml BD-I

Glucose:	4.5g
1M Tris-HCl pH8.0	12.5ml
0.5M EDTA pH8.0	10.0ml
Add ddH_2O	to 500ml

Filter sterilize
Store at 4°C

BD-II for Alkaline Lysis DNA Minipreps

ddH_2O	93ml
10M NaOH	2ml
20% SDS	5ml

BD-III for Alkaline Lysis DNA Minipreps

5M potassium acetate	60ml
Glacial acetic acid	11.5ml

Adjust pH to 4.8 (at least <5.4) with HCl (approx. 12ml concentrated HCl)
Add ddH_2O **to 100ml**
or

For 100 ml

Potassium acetate:	**29.4 g**
dH_2O:	**40 ml**
Glacial acetic acid:	**11.5 ml**

Adjust pH to 4.8 (at least <pH 5.2) with HCl (approx. 12ml concentrated HCl)
dH_2O **to 100 ml**
Filter sterilize
Store at 4°C

25x TAE (per liter)

Tris base	121g
Glacial acetic acid	28.55ml
0.5M EDTA (pH 8.0)	50ml
Add ddH_2O to	1000ml

10x TBE (per liter)

Tris base	108g

Boric acid 55g
0.5M EDTA (pH 8.0) 20ml
Add ddH_2O to 1000ml

10 M Ammonium Acetate (NH4C2H3O2)

1. Dissolve 771g ammonium acetate (m.w. = 77.1 g/mole) in 800 ml of H2O
2. Make final volume of 1 L with H2O
3. Sterilize by filtration and store at room temperature

1 M Dithiothreitol (DTT)

1. Dissolve 1.5g of DTT (DL-dithiothreitol, anhydrous m.w.=154.25) in 8 ml of deionized or distilled H20
2. Adjust volume to 10 ml and dispence into 1 ml aliquots
3. Store in dark, wrapped in foil at –20 C

0.5 EDTA (Disodium Ethylene Diamine Tetra-Acetate, pH 8.0) 500mM solution

1. Add 181.6 g of Na2EDTA2H2O to 800 ml H20
2. While stirring with a magnetic stirrer, adjust to pH 8.0 with pellets of NaOH (about 20 g NaOH
3. Sterilize by autoclaving and store at room temperature

Isopropyl-Beta-D-Thiogalactopyranoside (IPTG)

1. Dissolve 2 g of IPTG (m.w. = 238.3 g/mole) in 8 ml of distilled H2O
2. Adjust to10 ml with H20 and sterilize by filtration.
3. Dispense the solution into 1 ml aliquots and store at –20 C.

1 M Tris with Various pH Values

1. Dissolve 121.1 g of TRIS base in 800 ml H20
2. Adjust pH to the desired value by adding concentrated HCl.
3. Allow solution to cool to room temperature before making final adjustments to the ph
4. Adjust to 1 L with H20, dispense into, aliquots, sterile by autoclave, and store at room temperature

pH	HCL
7.4	70 ml
7.6	60 ml
8.0	42 ml

HEPES Bufffer (For 50 mM HEPES buffer @ pH 7.0)

1. Mix 25 mL of 200 mM HEPES (52.06 g/liter of HEPES Na salt), 11.8 of 100 mM NaOH
2. Add H2O to reach 100ml
3. Sterilize the solution by filtration

Phosphate Buffered Saline (PBS) (pH 7.2-7.4)

1. Dissolve the components in approximately 0.9 liters of H2O

2. Adjust pH to 7.2-7.4 with HCl and then adjust the final volume to 1 liter with H2O
3. Sterilize by autoclave for 20 minutes at 15 psi on liquid cycle

Component and final concentration	Amount to add per 1 liter
137 mM NaCl	8 g
2.7 mM KCl	200 mg
10 mM Na2HPO4O (dibasic, anhydrous)	1.44 g
2 mM KH2PO4O (monobasic, anhydrous)	240 mg
H20	To make 1 liter

Tris-EDTA (TE) (pH 7.4-8.0)

This standard buffer is used to resuspend and store DNA. It can be prepared by using stock solutions of 1 M Tris –HCL at pH values ranging from 7.4 to 8.0. Store at room temperature

Component and final concentration	Amount of stock to add per 100 ml
10 mM Tris-HCl	1 ml of 1 M (pH 7.4-8.0 at 25 C
1mM EDTA	200µl of 0.5 M (pH 8.0)
H20	98.9 ml

10X TE, pH 8 (100mM Tris-HCl, 10mM EDTA

	Final (1X)	Mix for 10X
Tris-HCl (pH 8.0)	10 mM	100ml of 1M
EDTA	1 mM	20 ml of 500 mM (pH 8.0)

Distilled H2O to 1 liter Sterilize by autoclaving. Store at room temperature

Proteins, Enzymes, and Antibiotics

20mg/ml Proteinase K

1. Add 200 mg of proteinase K to 15-ml polypropylene tube containing 9.5 ml H2O
2. To reduce denaturation, always add protein to an aqueous solution instead of adding aqueous solution to protein.
3. Gently rock until completely dissolved (no vortexing)
4. Adjust final volume to 10ml

10mg/ml Rnase A (Dnase-free)

1. Dissolve 10 mg of pancreatic Rnase A in 1 ml of 10mM sodium acetate (pH 5.0)
2. Place in boiling-water bath for 15 minutes to inactivate any contaminating Dnase
3. Adjust pH to 7.5 with 1 M Tris-HCl
4. When cool, aliquot and store at –20 C

Electrophoresis of DNA in Agarose Gels

50X TAE Buffer

This buffer does not have the buffering capacity of TBE buffer. The 1x TAE buffer (pH 8.1) is 40 mM Tris, 20 mM acetate, and 2 mM EDTA

Component and final concentration	Amount to add per 1 liter
2 M Tris base	242 g
1 M acetate	57.1 ml of glacial acetate acid (17.4 M)
100 mM EDTA	200ml of 0.5 M (pH 8.0)
H2O	To make 1 liter

5X TBE Buffer

TBE can be prepared as a 5X or 10X stock buffer, but the 10X stock buffer will precipitate during storage. Store at room temperature. 1X buffer (pH 8.3) is 89 mM Tris, 89 mM borate, and 2 mM EDTA.

Component and final concentration	Amount to add per 1 liter
445 mM Tris base	54 g
445 mM borate	27.5 g of boric acid
10 mM EDTA	20 ml of 0.5 M (pH 8.)
H2O	To make 1 liter

Sample Buffers for Protein Electrophoresis

2X Laemmli Sample Buffer Stock

1. Add 4 ml of 10% SDS, 2 ml of glycerol, and 1.2 ml of 1 M Tris (pH 6.8) to 2.8 ml of distilled H2O
2. Add bromophenol blue to 0.01% as a tracking dye.
3. Store at room temperature

20X SSC (Saline Sodium Citrate)

1. Dissolve 175.3 g of NaCl and 88.2 g of sodium citrate in 800 ml of H2o
2. Adjust pH to 7.0 with a few drops of a 10 N solution of NaOH
3. Adjust volume to 1 liter with H2O
4. Dispense in aliquots and autoclave.

20X SSPE (Saline, Sodium Phosphate, EDTA)

1. Dissolve 175.3 g of NaCl, 27.6 g NaH2PO4H2O, and 7.4 g of EDTA in 800 ml in H20
2. Adjust pH to 7.4 with NaOH (about 6.5 ml of a 10N solution
3. Adjust volume to 1 liter with H20
4. Dispense in aliquots and autoclave

GSB (6x DNA gel sample buffer)

SDS-PAGE Sample Buffer (for protein gels)

RNA Loading Solution

10x PCR Buffer (DMSO protocol)