

Williams' Lab Recipes

2-log DNA ladder

Recipe for one tube of 2-log DNA ladder (NEB; Cat. No. N3200L) has 500 ug of DNA ladder in 500 ul.

1. Add entire 500 ul of ladder to a 15 ml tube.
2. Add 750 ul of 10X Cresol Red loading to the 15 ml tube.
3. Add 3.75 ml of sterile milli-Q to the 15 ml tube, then mix, then spin down briefly
4. Aliquot in 250 ul or 500 ul aliquots to 1.5 mL tubes and store at -20C for long term storage.

* For a typical gel, loading 10 ul comes out to 1 ug of ladder, the amount shown on the NEB product web page.

2X General Footprint Buffer

(50 mL recipe; 50 mM HEPES pH 7.9, 100 mM KCl, 1 mM DTT, 12.5 mM MgCl₂, 0.05 mM EDTA, 17% Glycerol)

(1) To a 50 mL Conical Tube, add:

2.5 ml of 1.0 M HEPES (pH 7.9)

5 ml of 1.0 M KCl

50 µl of 1.0 M DTT

625 µl of 1.0 M MgCl₂

5 ml of 0.5 M EDTA

17 ml of 50% Glycerol

19.82 ml of milli-Q

(2) Cap conical tube and mix thoroughly.

(3) Filter sterilize buffer solution

(4) Label tube and store a -20°C (might be wise to divide into several smaller aliquots, 10-15mL)

1000x Ampicillin (sodium salt) 100mg/ml recipe

1. Measure out 1 g of Ampicillin tri hydrate
2. Add Milli-Q H₂O to 10 ml
3. Add ~.1 g of NaOH pellets (half pellet or more until Amp has dissolved)
 - You need to add an equal amount of NaOH moles to get Ampicillin into solution when using Ampicillin trihydrate. If Ampicillin is already a sodium salt, then disregard this step.
4. Sterilize solution by filtration through .2 micron filter on a syringe barrel
5. Aliquot and store @ -20° (stocks last about 1 year at -20) in 1.5 ml tubes

IPTG stock solution (0.1M)

1.2g IPTG

Add Milli-Q water to 50ml final volume.

Filter sterilize and store at 4°C.

Luria Broth (LB)

Tryptone	10 g
Yeast extract	5 g
Sodium chloride	10 g
dH ₂ O	to 1 L

Adjust pH to 7.5 with 5 N NaOH and autoclave.

LB-Amp Plates (make in large 2 L Erlenmeyer flask)

Tryptone	10 g
Yeast extract	5 g
Sodium chloride	10 g
Agar	20 g
dH ₂ O	to 1 L

Adjust pH to 7.5 with NaOH and autoclave.

When the solution cools to about 55°C, add 1mL of ampicillin (100 mg/mL) and pour (about 25 mL each) into clean plates, and allow to cool.

1X PBS, pH 7.4

NaCl	8 g
KCl	0.2 g
Na ₂ HPO ₄	1.44 g
KH ₂ PO ₄	0.24 g
dH ₂ O	to 1 L

Autoclave the solution.

10X PBS, 4 Liters

1. Fill large beaker with 3 L of Milli-Q ddH₂O
2. Add and mix:
 - 320 g NaCl
 - 8 g KCl
 - 57.6 g Na₂HPO₄ (dibasic) anhydrous
 - 9.6 g KH₂PO₄ (monobasic) anhydrous
3. pH to 7.4 using 5N NaOH
4. measure in graduated cylinder and add to carboy
5. add needed volume of Milli-Q ddH₂O to carboy to bring final volume to 4 L.
6. autoclave to sterilize

Sodium Azide (warning - very dangerous, take precautions)

To make a 10% stock solution of sodium azide, dissolve 10 g of sodium azide in 100 ml of distilled H₂O. Store at 4 degrees.

* Add Sodium Azide to a final concentration of 0.05% to prevent contamination: 5ul of 10% stock per 1 ml).

5X TBE buffer

54.0 g Tris base
27.5 g Boric acid
20 mL .05M EDTA
Fill with milli-Q for a total volume of 1000 mL

Autoclave on liquid cycle

TE (10 mM Tris-HCL and 1 mM EDTA pH 8)

1. Add 5 ml of 1M Tris-HCL (pH 7.4, 7.6, or even 8)
2. Add 1 ml of 0.5M EDTA pH 8
3. Add 494 ml of Milli-Q
4. Filter sterilize

1M Tris-HCL pH 9.5

Recipe for 500 ml

To 400 ml of milli-Q water add 60.57 g of Trizma (FW = 121.14 g Trizma) and dissolve.

Bring pH down to 9.5 (from 10.5) by drop-wise addition of concentrated HCl. Adjust volume of solution to 500 ml with milli-Q water and sterile filter of autoclave.

50X TAE Stock Solution

For each liter of solution:

242 g Tris Base (MW=121.1)

57.1 mL Glacial Acetic Acid (Glacial just means nearly pure – from Wikipedia)

100 mL 0.5 M EDTA pH 8

1. mix Tris with stir bar to dissolve in about 600 mL of ddH₂O.
2. add the EDTA and Acetic Acid.
3. bring final volume to 1 L with ddH₂O.
4. Do not autoclave, and store at room temperature.

20X TAE Stock Solution

For each liter of solution:

96.8 g Tris Base (MW=121.1) or Trizma Base (Sigma product)

22.84 mL Glacial Acetic Acid (Glacial just means nearly pure – from Wikipedia)

40 mL 0.5 M EDTA pH 8

1. mix Tris with stir bar to dissolve in about 600 mL of ddH₂O (Milli-Q).
2. add the EDTA and Acetic Acid.
3. bring final volume to 1 L with ddH₂O (Milli-Q).
4. Do not autoclave, and store at room temperature (Large Carboy).

X-Gal (2ml)

100mg 5-bromo-4-chloro-3-indolyl- β -D-galactoside

Dissolve in 2ml N,N'-dimethylformamide.

Cover with aluminum foil

and store at -20°C .

Last Updated On 9/12/12