Williams' Lab Recipes

ANTIBIOTICS

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

1. Measure out 1 g of Ampicillin tri hydrate
2. Add Milli-Q H_2O 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 trihyrdrate. 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 C (stocks last about 1 year at -20) in 1.5 ml tubes

1000x Kanamycin (50 mg/ml in H2O)

1. Measure 0.5 g of kanamycin sulfate (Sigma-Aldrich, K1876-5G) into a 15 ml falcon tube.
2. Add milli-Q to 10 ml
3. Sterilize solution by filtration through .2 micron filter on a syringe barrel
4. Aliquot and store @ -20C in 1.5 ml tubes

1000x Streptomycin (25 mg/ml in H2O)

1. Measure out .25 g of Streptomycin sulfate (Sigma-Aldrich, S6501-5G)
2. Add Milli-Q H_2O to 10 ml
3. Sterilize solution by filtration through .2 micron filter on a syringe barrel
4. Aliquot and store @ -20 C in 1.5 ml tubes

MEDIA FORMULATIONS

Luria Broth (LB)

Tryptone	10 g
Yeast extract	5 g
Sodium chloride	10 g
dH2O	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
dH2O	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.

2X YT Media (for protein expression in *E. coli*)

~900 mL ddH2O
16 g Bacto Tryptone
10 g Bacto Yeast Extract
5 g NaCl
Adjust pH to 7
Adjust volume to 1 L, divide volume into 500 mL bottles
Sterilize in autoclave

PROTEASE INHIBITORS

PMSF (100 mM in Isopropanol) *** Very Toxic ***

1. In 200 ml beaker, add 2 g of PMSF (174.19 g/mole; Fisher PI36978)
2. Working in hood, add stir bar and 114.94 ml of isopropanol
3. Using stirplate, mix until dissolved.
4. Aliquot into 2 ml tubes and store at -20 C.

DNA GEL ELECTROPHORESIS

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.

FOOTPRINTING AND GEL SHIFT ASSAYS

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)

1M HEPES, pH 7.9

HEPES has a FW of 238.30 g/mole (Sigma-Aldrich Cat.#H3375)

1. Dissolve 11.92 g HEPES in 40 ml milli-Q
2. Add 5 N NaOH with stirring until pH is 7.9
3. Adjust volume to 50 ml with milli-Q and sterile filter.
4. Store solution at 4C in the dark (cover with aluminum foil) to prevent break down into hydrogen peroxide

GST-Fusion Protein Elution Buffer (50 mL recipe)

(75 mM HEPES pH 7.9 (or 7.4), 150 mM NaCl, 10 mM (20 mM) reduced glutatione, 5 mM DTT, 0.1% Triton X-100)

1. Measure out 0.15366 (0.3073) g of reduced glutathione (307.32 g/mole; Cat.#G4251) and add to a 50 mL tube. (0.16 g is acceptable)
2. Add 3.75 mL of 1 M HEPES pH 7.9
3. Add 1.5 mL of 5 M NaCl
4. Add 250 uL of 1.0 M DTT (stored at -20C)
5. Add 500 uL of 10% Triton X-100
6. Add milli-Q (~44 mL) to bring volume to 50 mL
7. Filter sterilize after dissolving glutathione and aliquot 10mL into each of 5 15 mL tubes.
8. Store aliquots at -20C (Can be stored for at least 4 months without becoming significantly oxidized).

PROTEIN GELS

Coomassie Staining Solution (500 ml)

1 g Coomassie Brilliant Blue R-250
150 ml of methanol
50 ml of acetic acid

300 ml of milli-Q

1X SDS gel loading buffer (50 mM Tris-CL pH 6.8, 2% SDS, 0.1% bromophenol blue, 10% glycerol, 1% betamercaptoethanol - BME)

To make 40 ml in a 50 ml conical tube
Add 8 ml of 50% glycerol
2 ml of 1.0 M Tris pH 6.8
8 ml of 10% SDS solution
0.04 g of bromophenol blue (this is very blue, try not to get it on your clothes)

Store at room temperature
** Add BME to a small aliquot just before the loading buffer is used

1.5 M Tris pH 8.8 (for separating gels)

Recipe for 500ml
(1) To 400 ml of milli-Q water add 90.86g of Trizma Base (FW = 121.14 g Trizma) and dissolve
(2) Bring pH down to 8.8 (from 10.5) by drop-wise addition of concentrated HCl.
(3) Adjust volume of solution to 500 ml with milli-Q water and autoclave (or sterile filter).

1.0 M Tris pH 6.8 (for stacking gels)

Recipe for 500ml
(1) To 400 ml of milli-Q water add 60.57g of Trizma Base (FW = 121.14 g Trizma) and dissolve
(2) Bring pH down to 6.8 (from 10.5) by drop-wise addition of concentrated HCl.
(3) Adjust volume of solution to 500 ml with milli-Q water and autoclave (or sterile filter).

5X Tris-glycine running buffer

Add to 800 mL of milli-Q
15.1 g Tris base
94 g glycine
50 mL 10% SDS
After dissolving, bring volume up to 1000 mL with milli-Q

10X Transfer Buffer (for western blotting)

30.3 g Tris base
144 g glycine

Bring to 1 L in milli-Q

* for use mix 100 mL 10X Transfer buffer with 200 mL of methanol and 700 mL milli-Q

2X Sample Buffer (6% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.025% bromphenol blue, 125 mM Tris HCl pH 6.8, 2 mM EDTA)

To make 10 ml:
6.00 ml of 10% SDS solution
2.00 ml of 100% Glycerol
1.25 ml of 1 M Tris (pH 6.8)

40.0 ul of 0.5 M EDTA
250 ul of 1% bromophenol blue
460 ul of milli-Q

To make 40 ml:
24.00 ml of 10% SDS solution
8.00 ml of 100% Glycerol
5.00 ml of 1 M Tris (pH 6.8)

160.0 ul of 0.5 M EDTA
1.0 ml of 1% bromophenol blue
1.84 ml of milli-Q

******* Add 2-mercaptoethanol to 5% just prior to use *********
******* 1% bromophenol blue is 0.1 g in 10 ml of milli-Q *******

GENERAL USE SOLUTIONS

IPTG stock solution (0.1M)

1.2g IPTG
Add Milli-Q water to 50ml final volume.
Filter sterilize and store at 4°C.

PBS (1X), pH 7.4

NaCl	8 g
KCl	0.2 g
Na2HPO4	1.44 g
KH2PO4	0.24 g
dH2O	to 1 L

Autoclave the solution.

PBS (10X), 4 Liters

1. Fill large beaker with 3 L of Milli-Q ddH20
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 HaOH
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 H_2O. Store at 4 degrees.

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

STE buffer (10 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA)

To make 500 ml:

- 5.00 ml of Tris pH 8.0
- 15.0 mL of 5 M NaCl
- 500 ul of 0.5 M EDTA
- 479.5 ml of milli-Q

******* Store at 4 degrees to keep cold for protein purifications *********

TBE (5X) buffer

54.0 g Tris base
27.5 g Boric acid
20 mL 0.5M 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

TMNT Staining Buffer (100 mM Tris HCl pH 9.5, 50 mM MgCl2, 100 mM NaCl, and 0.1% Tween-20)

For 500 ml
1. add 50 mL of 1M Tris HCl pH 9.5
2. add 25 mL of 1M MgCl2
3. add 10 mL of 5M NaCl
4. add 500ul of Tween-20
5. add 414.5 ml of milli-Q

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 or autoclave.

TBST

10 mL Tris pH 8.0
30 mL 5 M Na Cl
1 mL Tween 20

Bring to 1 L in milli-Q

TAE (50X) 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.

TAE (20x) 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.

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