

# **R E C I P E S   I N D E X**

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## 2X SAMPLE BUFFER

### TO MAKE 8 ML

800 $\mu$ L 18 M $\Omega$  dH<sub>2</sub>O  
2.0mL 0.5M Tris (pH 6.8)  
1.6mL glycerol  
3.2mL 10% SDS  
0.5mL 0.1% Bromephenol Blue

### TO MAKE 50 ML

4.8mL 18 M $\Omega$  dH<sub>2</sub>O  
12.0mL 0.5M Tris (pH 6.8)  
9.6mL glycerol  
19.2mL 10% SDS  
3.0mL 0.1% Bromephenol Blue

1. Invert several times to mix thoroughly. Store at room temperature or 4°C
2. Add 50 $\mu$ L / mL 14.2M  $\beta$ -mercapto-ethanol right before using an aliquot of Sample Buffer

\* The buffer is only good for about 1 week after having added the  $\beta$ -mercapto-ethanol – lasts longer if stored at 4°C.

## COOMASSIE BLUE STAIN

### TO MAKE 1 LITER

500mL Methanol  
100mL Acetic Acid  
0.5g Coomassie Blue  
350mL 18 M $\Omega$  dH<sub>2</sub>O

### FINAL PERCENTAGES

50% Methanol  
10% Acetic Acid  
0.05% Coomassie Blue

Mix in 2L beaker on stir plate until all coomassie is dissolved. Takes 5-10 min.

## WATER EQUILIBRATED BUTANOL

35mL isobutyl alcohol  
15mL 18 M $\Omega$  dH<sub>2</sub>O

Mix in 50mL conical. Let sit about 1/2 hour before using so can separate.  
Use to remove bubbles from top of resolving gel when making Acrylamide Gels.

# AMPICILLIN

Want 50mg/mL stock → Mix 2.5g ampicillin with 50mL 18 MΩ dH2O

Store in 4°C with aluminum foil around 50mL conical. (Light sensitive)

# CHLORAMPHENICAL

Want 34mg/mL stock → Mix 1.7g chloram with 50mL ethanol

Store in -20°C with aluminum foil around 50mL conical. (Light sensitive)

# PEN/STREP (P/S)

*Makes 156mL 500X stock*

1.00 g Penicillin G \* (stored at room temp)

1.56 g Streptomycin sulfate (stored at 4°C)

Dissolve in 156mL 18 MΩ dH2O. Filter sterilize and aliquot: Draw up into a 60cc syringe. Put a filter on end and aliquot into 15mL tubes.

Store in -30°C freezer.

(Penicillin G is actually measured in units. 10,000,000 units is approximately 6.4g Original recipe called for 5,000,000 units penicillin + 5g streptomycin sulfate)

# 1.5M 10% SODIUM AZIDE

Makes 156mL 500X stock

5g sodium azide + 50mL 18 MΩ dH2O.

## LB

1. For 1 L of LB, mix the following ingredients in a 2L glass container with a stir bar until clear.

10 g Bacto-tryptone

5 g Bacto-Yeast Extract

5 g NaCl (sodium chloride)

1 L 18 MΩ dH<sub>2</sub>O

2. Add 200μL 5N NaOH (or 400μL 2.5M NaOH)
3. Aliquot as desired – screw on top or cover with aluminum foil, send out to be autoclaved.

## LB AGAR

1. For 1 L of LB Agar, mix the following ingredients in a glass container with a stir bar until everything is dissolved.

10 g Bacto-tryptone

5 g Bacto-Yeast Extract

5 g NaCl (sodium chloride)

1 L 18 MΩ dH<sub>2</sub>O

2. Add 200μL 5N NaOH (or 400μL 2.5M NaOH)
3. Aliquot 500mL into 1 L bottles.
4. Add 15g Bacto-Agar to each aliquot. Shake gently to mix as much as possible.
5. Screw on caps or cover with aluminum foil, label with date, send out to be autoclaved.

## POURING LB AGAR PLATES

1. Dissolve stored agar by micro waving until everything is melted. (Takes ~5 min.) Remove from microwave and shake a few times. **Use autoclave gloves!**
2. Let Agar cool to room temp. Add 2μL/mL of specified drugs.
3. Fill Petri dishes about 1/2 full.
4. Flame top of media quickly to pop the bubbles.
5. Let solidify, mark plates with designated drug symbols, & store upside down in cold room.

# 15% ACRYLAMIDE (SDS-PAGE) GELS

1. Rinse caster, plates, & spacers with dH<sub>2</sub>O. Need 12 front & back plates & 13 spacers.
2. Load plates: spacer, front plate, back plate. Start with a spacer and end with a spacer. Clamp caster side tight. Test for leaks by filling caster with dH<sub>2</sub>O.
3. Make 1.3mL 10% APS – suspend 0.13g APS with 1.3mL 18 MΩ dH<sub>2</sub>O
4. Prepare RESOLVING GEL mixture in 100mL-graduated cylinder.
  - 40mL Resolving Gel Buffer – stored at room temp.
  - 40mL 30% Acrylamide – stored at 4°
  - 800μL 10% APS
  - 30μL TEMED

**Add the APS & TEMED quickly. Invert a few times.**
5. Pour evenly over plates. (Should have ~ 30mL left of mixture) Avoid bubbles. Leave about 1/2” below top of the small front plates.
6. Pipette 200μL butanol over each gel to pop any bubbles.
7. Let polymerize about 1 hour. Will know that the gel has solidified by looking at the leftover gel.
8. Pour off butanol.
9. Prepare STACKING GEL mixture in a 100mL-graduated cylinder.
  - 45 mL Stacking Gel Buffer – stored at room temp.
  - 9 mL 30% Acrylamide – stored at 4° (want 5% final Acrylamide conc.)
  - 500 μL 10% APS
  - 33 μL TEMED

**Add the APS & TEMED quickly. Invert a few times.**
10. Pour evenly into caster to the top. Avoid bubbles.
11. Insert combs, pour a little more gel on top, & allow to polymerize for ~ 1/2 hr (until hard.)
12. Remove gels from caster. Take out spacers, but KEEP COMBS IN. Wrap gels in packs of 6 in paper towels. Wet paper towels with dH<sub>2</sub>O & wrap in saran wrap. Label with type of gel, date, & number of wells.
13. Store at 4°C – gels will last about 1 month.

**To Clean Plates** – wash carefully WITHOUT soap in regular water. Rinse with house dH<sub>2</sub>O. Let dry.  
KDG, Robinson Lab

## **RESOLVING GEL BUFFER**

Mix ingredients and store in a 500mL bottle at room temperature. (Makes ~500mL)

228mL 18 M $\Omega$  dH<sub>2</sub>O

250mL 1.5M Tris (pH 8.8)

10mL 10% SDS

## **STACKING GEL BUFFER**

Mix ingredients and store in a 500mL bottle at room temperature. (Makes ~400mL)

277.5mL 18 M $\Omega$  dH<sub>2</sub>O

125.0mL 0.5M Tris (pH 6.8)

5.0mL 10% SDS

## TB

1. For 500mL, mix the following ingredients in a glass container with a stir bar until all the salts are dissolved. Can heat the mixture a little if necessary to help salts dissolve.

1.51g PIPES

1.10g CaCl<sub>2</sub>

9.32g KCl

*Salts will not completely dissolve until increase pH using 10M KOH.*

~450mL 18 MΩ dH<sub>2</sub>O

2. Adjust pH to 6.7 using KOH. Add slowly because will jump when gets close.
3. Add 5.44g MnCl<sub>2</sub> (manganese chloride). Mix until dissolved.
4. Adjust volume to 500mL with 18 MΩ dH<sub>2</sub>O .
5. Filter sterilize & store in 4°C fridge.

## SOB

1. For 1L, mix the following ingredients in a glass container with a stir bar until dissolved.

20.0g Bacto-tryptone

5.0g Bacto-yeast Extract

0.5g NaCl

~950mL 18 MΩ dH<sub>2</sub>O

2. Add 833μL 3M KCl.
3. Adjust pH to 7.0 with 5M NaOH or HCl.
4. Adjust volume to 1L with 18 MΩ dH<sub>2</sub>O.
5. Aliquot 250mL into 2L beakers. Label with date and cover with foam stoppers, aluminum foil, & autoclave tape.
6. Send out to be autoclaved. Let cool before inoculate!
7. **BEFORE INOCULATE SOB ALIQUOTS, ADD 5ML 2M MGCL<sub>2</sub> OR 10ML 1M MGCL<sub>2</sub>**

**To make 2M MgCl<sub>2</sub>:** Add 19g MgCl<sub>2</sub> to 90mL 18 MΩ dH<sub>2</sub>O - mix until dissolved, then raise volume to 100mL with 18 MΩ dH<sub>2</sub>O. Send out to be autoclaved before use.

# F-ACTIN

1. Activate an aliquot of 10X F-Actin Buffer by mixing the following:

500 $\mu$ L 10X F buffer

10 $\mu$ L 0.1M ATP  $\rightarrow$  2mM final conc.

5 $\mu$ L 1.0M DTT  $\rightarrow$  10mM final conc.

2. Mix the following by pipeting without creating bubbles:

## FOR 126 $\mu$ M G-ACTIN

50.0 $\mu$ L 126 $\mu$ M G-actin (stored at -80 $^{\circ}$ C)

5.5 $\mu$ L 10X Mg Exchange Buffer

6.2 $\mu$ L 10X F-actin Buffer

**Final Vol. = 61.2 $\mu$ L**

**Final [F-Actin] = 102 $\mu$ M**

## FOR Q $\mu$ M G-ACTIN

w  $\mu$ L Q $\mu$ M G-Actin

y  $\mu$ L 10X Mg Exchange Buffer

z  $\mu$ L 10X F-Actin Buffer

**y = w / 9      z = (w + y) / 9**

**Final Vol. = w + y + z**

**Final [F-Actin] = (w \* Q) / (w + y + z)**

3. Let the mixture polymerize at room temp for 30 min. Should see little air bubbles getting trapped in the mixture as it thickens up into a gel like substance.
4. Store on wet ice in cold room. F-actin is only good for a few days. Make sure mix well before using to break up long actin filaments.

# 1X F BUFFER

Mix 100 $\mu$ L 10X F-Buffer with 900 $\mu$ L 18 M $\Omega$  dH<sub>2</sub>O

# 10X F-BUFFER

## To make 14 mL

2.3 mL	3M KCl
140µL	1M MgCl <sub>2</sub>
280µL	0.5M EGTA
2.1mL	1M Imidazole pH 7.0

## Final Concentrations

500mM KCl
10mM MgCl <sub>2</sub>
10mM EGTA
150mM Imidazole

Add 18 MΩ dH<sub>2</sub>O to raise volume to 14mL. Store at room temp. Add ATP & DTT to small aliquots as needed.

# 10X MG EXCHANGE BUFFER

## To make 14 mL

84µL	0.5M EGTA
28µL	1M MgCl <sub>2</sub>
280µL	0.5M Tris pH 7.5

## Final Concentrations

3mM EGTA
2mM MgCl <sub>2</sub>
10mM Tris pH 7.5

Add 18 MΩ dH<sub>2</sub>O to raise volume to 14mL. Store at room temp.

## 2L 1X HL-5 MEDIA

1. Pour ~ 1,200mL 18 MΩ dH<sub>2</sub>O into a glass container. Dissolve the following while stirring with a stir bar:

20.0g	Proteose Peptone	0.17g	Na <sub>2</sub> HPO <sub>4</sub> -7H <sub>2</sub> O
5.2g	Bacto-Yeast Extract	0.14g	KH <sub>2</sub> PO <sub>4</sub>
4.0g	Glucose		

2. Adjust pH to 6.5 using 1M K<sub>2</sub>HPO<sub>4</sub> to make more basic or 1M KH<sub>2</sub>PO<sub>4</sub> to make more acidic.
3. Add 18 MΩ dH<sub>2</sub>O to bring final vol. to 2L. Send out to be autoclaved for 24 min.

## 2L 1.5X HL-5 MEDIA

1. Pour ~ 1200mL 18 MΩ dH<sub>2</sub>O into a glass container. Dissolve the following while stirring with a stir bar:

30.0g	Proteose Peptone	0.255g	Na <sub>2</sub> HPO <sub>4</sub> -7H <sub>2</sub> O
7.8g	Bacto-Yeast Extract	0.255g	KH <sub>2</sub> PO <sub>4</sub>
6.0g	Glucose		

2. Adjust pH to 6.5 using 1M K<sub>2</sub>HPO<sub>4</sub> to make more basic or 1M KH<sub>2</sub>PO<sub>4</sub> to make more acidic.
3. Add 18 MΩ dH<sub>2</sub>O to bring final vol. to 2L. Send out to be autoclaved for 24 min.

## 1L BIS-TRIS HL-5 PH 6.7

1. Pour ~ 600mL 18 MΩ dH<sub>2</sub>O into a glass container. Dissolve the following while stirring with a stir bar:

10.0g	Proteose Peptone	20mL	0.5M Bis-Tris-Cl pH 6.7 (want 10mM final)
2.6g	Bacto-Yeast Extract		(To make 0.5M Bis-Tris-Cl – 20.92g Bis-Tris
2.0g	Glucose		dissolved in 200mL dH <sub>2</sub> O. Use HCl to get 6.7pH)

2. Double check pH and adjust as necessary with HCl.
3. Add 18 MΩ dH<sub>2</sub>O to bring final vol. to 1L. Send out to be autoclaved for 24 min.

## HAN'S ENRICHED MEDIA

500mL 1X HL-5	<b>OR</b>	500mL 1.5X HL-5
75mL 5X HL-5		43mL FM
50mL FM		

# 1L LOW FLUORESCENT MEDIA FOR DICTY

1. Pour ~ 600mL 18 MΩ dH<sub>2</sub>O into a glass container. Dissolve the following while stirring with a stir bar:

3.85g	Glucose	1.200g	Na <sub>2</sub> HPO <sub>4</sub> -12H <sub>2</sub> O
1.78g	Proteose Peptone	0.485g	KH <sub>2</sub> PO <sub>4</sub>
0.45g	Bacto-Yeast Extract		

2. Bring volume up to 1L. Autoclave.

(Taro Uyeda, J. Cell Sci. 115, 2241-2251, 2002)

## MEDIA FOR DICTY CELLS

1. Add 43mL FM to 500mL 1.5X HL-5 to make Enriched Media
2. Add only 1mL Penn Strep to all bottles of Enriched for parent strains: ORFT & 11-5.1.
3. Add 1mL Penn Strep + 200μL 10mg/mL Blasticidin to 500mL Enriched Media so the final concentration of Blast is 4μg/mL.
4. Add 1mL Penn Strep & 81.6μL 100mg/mL G418 to 500mL Enriched Media so the final concentration of G418 is 15μg/mL.

## KLEBSIELLA PREP

1. Add 2mL K.a. to 200mL LB (no drug selection) and incubate overnight in 37°C.
2. Autoclave if going to use to feed dicty. Or use live if plating K.a. lawns on SM-5 plates.

## 2L FM

1. FM Medium is stored in 4°C fridge.
2. Mix 38g FM with ~1,500 mL 18 MΩ dH<sub>2</sub>O. Dissolve by stirring rapidly. May not get all into solution. Raise volume to 2L and stir more – may help get into solution better.
3. Aliquot into 500mL bottles. Cover bottles with foil because FM is sensitive to light.
4. Label and send out to be autoclaved for 24 min.

## 1L SM-5

1. Dissolve the following in about ~600mL 18 MΩ dH<sub>2</sub>O:

2.0 g	Bacto Tryptone	1.9 g	Potassium Phosphate Monobasic (KH <sub>2</sub> PO <sub>4</sub> )
2.0 g	Bacto Yeast Extract	1.0 g	Potassium Phosphate Dibasic (K <sub>2</sub> HPO <sub>4</sub> )
2.0 g	Glucose	0.2 g	Magnesium sulfate Heptahydrate (MgSO <sub>4</sub> -7H <sub>2</sub> O)

2. Adjust pH to 6.4 with 2.5M NaOH if too acidic or 1M KH<sub>2</sub>PO<sub>4</sub> if too basic.
3. Aliquot either 1L into a 2L bottle or 500mL's into 2 1L bottles.
4. Add 10g Bacto-Agar per 500mL. (so if 1L in 2L bottle, add 20g Bacto-Agar)
5. Label and send out to be autoclaved for 27 min.

( 1 Liter makes about 30 plates )

## TO POUR PLATES

1. Let media cool to touch – can use a 60°C hot water bath to cool when media returns from autoclave. Make sure all media is dissolved (not solidified on bottom of bottle).
2. Flame mouth of flask and pour media into plates. (Keep covers on plates between steps.)
3. Quickly flame top of media to remove any bubbles. Set aside to cool. (Takes several hours)
4. Once all moisture is gone from plate cover, turn plates upside down, label and store in 4°C cold room.

# LYSIS BUFFER

1. Mix the following ingredients – make 30mL per every 1L of culture started with:

		<b>For 30mL</b>	<b>For 500mL</b>
20 mM NaCl	(stock is 5.0M)	120 $\mu$ L NaCl	2.0 mL NaCl
10 mM Hepes pH 7.1	(stock is 1.5M)	200 $\mu$ L Hepes pH 7.1	3.3 mL Hepes
1 mM EDTA	(stock is 0.5M)	600 $\mu$ L EDTA	1.0 mL EDTA
1 mM EGTA	(stock is 0.5M)	600 $\mu$ L EGTA	1.0 mL EGTA

Add enough 18 M $\Omega$  dH<sub>2</sub>O to bring the volumes up to specified amounts.

2. Add the protease inhibitor cocktails. Make protease inhibitors as seen on next page.

**\* Add 10mM  $\beta$ ME into lysis buffer when making N Cys proteins.  
Add 0.7 $\mu$ L 14.2M  $\beta$ ME stock / mL of lysis buffer**

# DIALYSIS BUFFER

Notes: Makes 2L

1. Combine the following in a 2L graduated cylinder:

	<b>FINAL CONCENTRATIONS</b>
13.3 mL 1.5M Hepes (pH 7.1)	10mM Hepes
800.0 $\mu$ L 5.0M NaCl (sodium chloride)	2mM NaCl
1.3 mL 1.5M NaN <sub>3</sub> (sodium azide)	1mM NaN <sub>3</sub>

**COVER TOP WITH PARAFILM & INVERT A FEW TIMES TO MIX.**

# PROTEASE INHIBITORS

INDIVIDUAL INHIBITORS	[ STOCK ]	[ FOLD ]	DISSOLVE IN	STORE AT
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Prepare the first 3 individually.

PMSF	100 mM	1,000X	EtOH	-20°C
TLCK	15 mM	100X	1mM HCl	4°C
Aprotinin**	10 mg / mL	10,000X	EtOH	4°C
** Dilute in pH 6-8 buffer & avoid refreezing				

Prepares the last 5 as a cocktail in EtOH – store at -20°C

					RECIPE FOR 20mL
Leupeptin	500 µg / mL	100X	EtOH	-20°C	10 mg
Benzamidine	100 µg / mL	100X	EtOH	4°C	2 mg
Pepstatin A	200 µg / mL	100X	EtOH	4°C	4 mg
TAME	10 mg / mL	100X	EtOH	-20°C	200 mg
TPCK	8 mg / mL	100X	EtOH	4°C	160 mg
Add ~15mL EtOH & dissolve inhibitors. Bring volume up to 20mL with EtOH.					

# ACETONE POWDER PREP

Notes: Fill out an Acetone Powder Prep Worksheet & file in your notebook.

Prep – can do this up to a week before:

1. Sterilize cheesecloth by boiling in dH<sub>2</sub>O for 20 min. Drain and chill at 4°C until ready to use.
2. Prepare Solutions and store at 4°C until ready to use. Need:

1L        0.1M KCl, 0.15M KHPO<sub>4</sub> (pH 6.5)  
          add 685mL 1M monobasic (136.1g/L)  
              315mL 1M dibasic (174.2g/L)

2L        0.05M NaHCO<sub>3</sub> → add 4.2g/L

1L        1mM EDTA (pH 7.0) → add 3.7g/L

5L        100% Acetone

3. Rinse muscle with dH<sub>2</sub>O and chill on ice. Mince in pre-chilled meat grinder.
4. *KCL Extraction*: Stir 10 min. in 1L ice-cold 0.1M KCl, 0.15M KHPO<sub>4</sub> (pH 6.5). Filter through 4 layers of cheesecloth. Save the powder and discard the sup.
5. *Bicarb Extraction*: Stir 5 min. at 4°C in 2L prechilled 0.05M NaHCO<sub>3</sub>. Filter through 4 layers of cheesecloth. Save the powder and discard the sup.
6. *EDTA Extraction*: Stir 10 min. at 4°C in 1L 1mM EDTA, pH 7.0. Filter through 4 layers of cheesecloth. Save the powder and discard the sup.
7. *H<sub>2</sub>O Extraction*: Stir 5 min. at 4°C in 2L 18 MΩ dH<sub>2</sub>O. Filter through 4 layers of cheesecloth. Save the powder and discard the sup.
8. *Acetone Extraction*: Stir 10 min. at 25°C in 1L Acetone. (Acetone must be chilled below 20°C) After each extraction, filter through 4 layers of cheesecloth. Save the powder and discard the sup.  
**Repeat 4 more times.**
9. Air-dry acetone overnight in hood to generate acetone powder. Store powder in 50mL conicals in a box in -20°C. Label with batch #, date, grams in each conical, and initials.

# G-ACTIN PREP

Notes: Use ground actin powder stored in 50mL conicals stored in -20°C freezer.  
Fill out an Actin Prep Worksheet as make actin & file in your notebook.

## RINSE ALL GLASSWARE AND TOOLS WITH 18 MΩ DH2O BEFORE USE

Prep – can do this up to a week before:

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1. Sterilize cheesecloth by boiling in dH2O for 20 min. Drain and chill at 4°C until ready to use.
2. Prepare Buffer G and store at 4°C until ready to use.

	<b>For 2L</b>
2mM Tris Base (0.242 g/L)	0.48 g Tris Base
0.2mM NaATP (0.11 g/L)	0.22 g Na <sub>2</sub> ATP (ATP-stored in -20°C)
0.2mM 1M CaCl <sub>2</sub> stock (200 μL/L)	400 μL 1M CaCl <sub>2</sub>
0.005% 1.54M 10% Azide (500 μL/L)	1000 μL 1.54M 10% Sodium Azide
	Enough 18 MΩ dH2O to bring to 2L

3. Set pH to 8.0 using 10M HCl – ADD SLOWLY (takes ~160μL)
- 

4. Just before use, add stock βME (14.3M) to Buffer G so that have a final concentration of 0.5mM.

a.  $(14300\text{mM})(x) = (0.5\text{mM})(\text{Vol}_{\text{Buffer G}})$                       Add 35μL βME per liter of Buffer G

5. Weigh out powder. Usually use about 10g.
6. *Extraction 1: In a cold room*, pour powder into a beaker with stir bar and add **20mL Buffer G per gram** of powder. Let spin for 30 min. Pour off supernatant through sterilized cheesecloth into clean beaker. Minimize powder loss.
7. *Extraction 2: Remain in cold room* and add same amount of Buffer G to remaining powder and spin for another 5 min. Pour off supernatant through cheesecloth and squeeze cloth to get all supernatant. Discard powder and save sup.
8. Pour supernatant into clear 70mL centrifuge conicals (ones with the red metal caps). Weight cap, sample, and tube together to balance out tubes.
9. Spin extracts in Type 45Ti rotor (Jensen Lab) for 30 min. at 20K rpm (46000 x g)
10. Decant supernatant, measure & record the volume.
11. Polymerize actin by adding the following. Stir at very low speed for 5 min. at room temp. Then remove from stir plate and incubate at room temp for another 55min.

KCl to final concentration of 50.0mM    (Vol.)(0.0167) = \_\_\_\_ mL 3M KCl stock  
MgCl<sub>2</sub> to final concentration of 2.0mM    (Vol.)(0.002) = \_\_\_\_ mL 1M NaCl<sub>2</sub> stock

- ATP to final concentration of 1.0mM (Vol.)(0.605) = \_\_\_\_ g ATP powder
12. Raise KCl to 0.6M & stir for 15 min at room temp.  
(Vol.)(0.041) = \_\_\_\_ g solid KCL
  13. Spin at 42K rpm (205,000 x g) for 60 min in Type 45Ti rotor. (Only spin at this speed if tubes are completely full – cut back speed if not full.)
  14. Decant supernatant and spin again at 42K for 60 min in Type 45Ti rotor.
  15. Take pellet & add 1 mL Buffer G per tube. Incubate 15 min on ice.
  16. Decant and discard second supernatant. Take pellet and add 0.7 mL Buffer G per tube. Incubate on ice for 15 min.
  17. Rinse homogenizer with 18 MΩ dH2O thoroughly. Combine “resuspended” pellets and transfer to a homogenizer. Homogenize in as small a volume as possible.
  18. Dialyze against 2L of Buffer-G overnight in cold room on stir plate. Change buffer in morning and change buffer again at end of day and leave overnight.

\*Use 2 extra changes and leave out βME for all dialysis if planning to label with pyrene.

19. Spin at 95,000 x g for 20 min.
20. Recover and recombine supernatants. Determine the actin concentration using  $\lambda$  function on the spectrometer. Dilute 4 μL actin in 396μL 18 MΩ dH2O for a 100-fold dilution. Dilute 4μL actin dialysis buffer in 396μL 18 MΩ dH2O for a 100-fold dilution. Use 290 as the wavelength and blank against the diluted dialysis buffer.

Concentration (mg/mL) = (OD290 / 0.62) x dilution factor

( \_\_\_\_\_ / 0.62) \* \_\_\_\_\_ 100 = \_\_\_\_\_ mg/mL

Actin = 24mM = 1 mg/mL therefore, \_\_\_\_\_ mg/mL \* 24mM = \_\_\_\_\_ mM

21. Aliquot actin. Flash freeze in liquid nitrogen and store in -80°C freezer.

# 10X PBS BUFFER

## *To make 1 Liter*

1. Dissolve the following in ~800mL 18 MΩ dH<sub>2</sub>O. May need to heat a little to dissolve completely.

80g NaCl                      14.4g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (dibasic)

2g KCl                        2.4g KH<sub>2</sub>PO<sub>4</sub> (monobasic)

2. Adjust pH to 7.4 with HCl or NaOH.
3. Raise volume to 1 liter.
4. Transfer to a 1L bottle, label and autoclave.

# 0.5M PIPES BUFFER

## *To make 200mL*

1. Dissolve 30.2g PIPES in ~140mL 18 MΩ dH<sub>2</sub>O. It will be a thick, white, opaque liquid.

$$\text{FW} = 302.4\text{g/mol} \quad (302.4\text{g/mol})(0.5\text{M})(0.2\text{L}) = 30.2\text{g}$$

2. Bring pH up to 6 using 5M NaOH. The liquid will become clear as most of the PIPES goes into solution. There will still be some powder not in solution.
3. Start adding 5M NaOH more slowly – about 10μL at a time. Let the solution equilibrate each time because every time add base, more PIPES goes into solution and lowers pH. Want **final pH of 6.5**, but as soon as rest of PIPES goes into solution, the pH will jump up much faster.
4. Raise volume to 200mL. Store at room temp in 200mL bottle. Label with date, pH, and write “nonhazardous buffer” at bottom of label.

# 50X TAE BUFFER

## *To make 1 Liter*

1. Dissolve the following in ~800mL 18 MΩ dH<sub>2</sub>O. May need to heat a little to dissolve completely.

242.0 g Tris Base

57.1 mL glacial acetic acid

100.0 mL 0.5M EDTA (pH 8.0)

2. Raise volume to 1L and store. Dilute 50-fold to use as DNA gel electrophoresis buffer.

# 10X ELECTROPHORESIS BUFFER

30 g/L Tris Base

144 g/L Glycine

10 g/L SDS (or 100mL/L 10% SDS or 50mL/L 20% SDS)

## SOLUTION I

*Stock stored at 4 °C*

*(Need 100  $\mu$ L per mini-prep culture.)*

1. To make 200mL, dissolve the following in ~100mL 18 M $\Omega$  dH<sub>2</sub>O.

50mM glucose	= 1.802g glucose
25mM Tris (pH 7.5)	= 5mL 1M Tris (pH 7.5)
10mM EDTA	= 4mL 0.5M EDTA

2. Raise volume to 200mL. Autoclave and store at 4°C.

## SOLUTION II

*Make small amounts as needed – does not store well*

*(Need 200  $\mu$ L per mini-prep culture.)*

1. Calculate amount of stocks so that have final concentrations listed below.

0.2M NaOH  
1 % SDS

2. Use immediately. Do not use if older than 1 week.

## SOLUTION III

*Make 200mL and store at room temperature.*

*(Need 150  $\mu$ L per mini-prep culture.)*

1. Mix the following:

120mL 5M potassium acetate  
23mL glacial acetic acid  
57mL 18 M $\Omega$  dH<sub>2</sub>O

2. Result = 3M potassium & 5M acetate. Store at room temperature.

# SOUTHERN HYBRIDIZATION BUFFERS

## Depurination Buffer

0.25N HCl

## Denaturing Buffer

1.5M NaCl

0.5N NaOH

## Neutralization Buffer

1M Tris pH7.4

1.5M NaCl

## Church's Hybridization Buffer

0.25M NaPO<sub>4</sub> pH7.0

7% SDS

1g BSA

1mM EDTA

50mLs 0.5M NaPO<sub>4</sub> pH7.0

35mLs 20% SDS

1g BSA

200uL 0.5M EDTA

## Oligo labeling STOP buffer

50mM Tris pH7.5

2.8M NH<sub>4</sub>OAc

10mM EDTA

200ug/mL tRNA

## Oligo labeling buffer

### Solution O

1.25M Tris pH8.0

0.125M MgCl<sub>2</sub>

} Store at 4C

### Solution A

1mL of solution O buffer and 5uL of each 100mM dATP

dTTP

dGTP

store at -20C

### Solution B

2M Hepes pH6.6 with NaOH store at 4C

### Solution C

Hexadeoxyribonucleotide in TE at 90 OD/mL

Store at -20C

## \*To make 5X OLB:

Mix solutions in the following ratio A:B:C:H<sub>2</sub>O=10:25:11:4

# NORTHERN HYBRIDIZATION BUFFERS

## 10X MOPS

0.2M MOPS	41.8g
50mM NaAc	4.1g
10mM EDTA	3.7g
H2O	800 mL

Adjust pH 7.0 with ~8mL 10M NaOH

H2O to 1L and divide into 2 500mL aliquots

AUTOCLAVE- will turn yellow

## DEPC-treated dH2O

Add 18mΩ dH2O to Rnase-free glass bottles.

Add DEPCI to 0.01% (v/v)

Let stand O/N and autoclave

## DEPC-treated TE (Tris-EDTA)

	Stock	250mL
10mM Tris pH7.6	1M	2.5mL
1mM EDTA	0.5M	0.5mL
DEPCI-H2O		247mL
AUTOCLAVE		

## DEPC-treated formaldehyde gel-loading buffer

	Stock	3mL
50% glycerol	100%	1.5mL
1mM EDTA (pH 8.0)	0.5M	6μL
0.25% bromophenol blue		0.0075g
0.25% xylene cynaol FF		0.0075g
DEPCI-H2O to 3 mL		

## 10 mg/mL DEPC-treated ethidium bromide

	6mL
Ammonium Acetate	0.0463g
Ethidium Bromide	0.006g
DEPC-H2O to 6 mL	

## 20x SSC Buffer

NaCl	175.3g
sodium citrate	88.2g
DEPC-H2O	800mL

Adjust the pH to 7.0 with a few drops of 10N NaOH. Adjust to 1L, AUTOCLAVE.

# NORTHERN HYBRIDIZATION BUFFERS, CONTINUED

## 20% SDS

**Caution:** Wear goggles and face mask when measuring SDS and clean surfaces when finished.

SDS powder	100g
H <sub>2</sub> O	300mL

Heat to 68C to assist in dissolving. Adjust to 500 mL and aliquot. No need to sterilize 20% SDS.

## 0.5M sodium phosphate buffer pH 7.0

*First make:*

1L, 1M Na<sub>2</sub>HPO<sub>4</sub> (dibasic)      FW=268.07g

1L, 1M NaH<sub>2</sub>PO<sub>4</sub> (monobasic)      FW=137.99g

Bring each to 1L and AUTOCLAVE

### 1M sodium phosphate buffer pH 7.0

390mL NaH<sub>2</sub>PO<sub>4</sub> (monobasic)

610mL Na<sub>2</sub>HPO<sub>4</sub> (dibasic)

AUTOCLAVE

Add 250mL of 1M sodium phosphate buffer pH7.0 and bring to 500mL with 18MΩ dH<sub>2</sub>O.

AUTOCLAVE