

MORIMOTO LAB BUFFER AND SOLUTION RECIPES

30% Acrylamide	100ml 29g 2X Acrylamide 1g <i>N,N'</i> -methylenebisacrylamide Add ~300ml ddH <sub>2</sub> O. Heat to 37°C to dissolve chemicals. Adjust final volume to 500ml with ddH <sub>2</sub> O.	Serva (10675) Serva (29195)
Ampicillin 100mg/ml	50ml: 5g Ampicillin Add ddH <sub>2</sub> O to 50ml. Sterilize using a 0.22 μm filter. Aliquot and store at -20°C. (Use at 100μg/ml).	Sigma (A-9518)
10% APS	10ml: 1g ammonium persulfate ddH <sub>2</sub> O to 10ml This solution may be stored at 4°C for several weeks.	BioRad (161-0700)
Buffer C	20mM HEPES pH 7.9 25% (v/v) glycerol 0.42 M NaCl 1.5 mM MgCl <sub>2</sub> 0.2 mM EDTA --add 0.5mM DTT before use Store buffer without DTT at 4°C.	Sigma (H-3375) Fisher (G33-500) Sigma (S-9625) Sigma (M-9272) Sigma (ED2SS)
1M CaCl <sub>2</sub>	200ml 54g CaCl <sub>2</sub> • 2H <sub>2</sub> O Dissolve in 200ml ddH <sub>2</sub> O. Sterilize by filtration through a 0.22μm filter.	Sigma (C-5080)
Chloramphenicol 34mg/ml	500ml: 17g Chloramphenicol ethanol to 500ml No need to filter sterilize. Store at -20°C.	Sigma (C-0378)

DNase I (DPRF) 5 mg/ml	10ml: (new vial contains $\geq 10,000$ units Dnase I lyophilized) Dissolve the entire 10,000 unit vial in 1.0 ml H <sub>2</sub> O (provides the equivalent of a 5 mg/ml solution). Make 20 $\mu$ l aliquots and store at $-20^{\circ}\text{C}$ .	Worthington Biomedical (LS06333)
1M DTT (Dithiothreitol)	20ml: 3.09 g DTT Dissolve in 20ml 0.01M NaOAc (pH5.2) Sterilize by filtration Dispense into aliquots and store at $-20^{\circ}\text{C}$	Roche (100 034) Sigma (S-8750)
0.5 M EDTA pH 8.0	1 liter: 186.1 g EDTA • 2H <sub>2</sub> O Add dH <sub>2</sub> O to about ~800 ml. Stir vigorously. Adjust pH to 8.0 with about 20 g NaOH pellets ( <i>solution will not dissolve until the pH is approximately 8.0 by the addition of NaOH; usually takes ~19-25g NaOH pellets</i> ). Bring volume up to 1 L. Use vacuum bottle-top filter to remove any particles. Autoclave and store at room temp.	Sigma (ED2SS)
Ethidium bromide (10mg/ml)	10ml: 100 mg Ethidium bromide add ddH <sub>2</sub> O to 10ml Stir on a magnetic stirrer for several hours to ensure that dye has completely dissolved. Store in a dark bottle at room temp.  <i>Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when handling solutions containing the dye and a mask should be worn when weighing it out.</i>	Sigma (E-8751)
5x Gel Loading Dye (for DNA gels)	10ml 5x buffer: 5.0 ml glycerol                      50% glycerol 5 ml 5xTBE                            2.5xTBE 25 mg Bromophenol Blue        0.25% Bromophenol Blue 25 mg Xylene cyanol FF        0.25% Xylenephenol Blue  Bromophenol Blue migrates at ~300bp dsDNA (linear) Xylene cyanol blue migrates at ~4000bp dsDNA (linear)	

IPTG	10ml: 2 g IPTG add to ddH <sub>2</sub> O 8ml Adjust final volume to 10ml with ddH <sub>2</sub> O. Sterilize using a 0.22 μm filter. Store at -20°C.	Sigma (I-5502)																					
Kanamycin (10mg/ml)	10ml: 100 mg Kanamycin A add ddH <sub>2</sub> O to 10 ml Sterilize using a 0.22 μm filter. Store at -20°C. (Use at 30μg/ml)	Sigma (K-1876)																					
Laemmli sample buffer	<table border="1"> <tr> <td><u>2x</u></td> <td><u>5x</u></td> <td><u>16 ml:</u></td> </tr> <tr> <td>10.4 ml</td> <td>6.8 ml</td> <td>ddH<sub>2</sub>O</td> </tr> <tr> <td>1.2 ml</td> <td>2.0 ml</td> <td>0.5M Tris pH 6.8</td> </tr> <tr> <td>1.9 ml</td> <td>3.2 ml</td> <td>Glycerol</td> </tr> <tr> <td>1.0 ml</td> <td>1.6 ml</td> <td>20%SDS</td> </tr> <tr> <td>0.5 ml</td> <td>0.8 ml</td> <td>β-mercaptoethanol</td> </tr> <tr> <td>1.0 ml</td> <td>1.6 ml</td> <td>1% Bromophenol blue</td> </tr> </table>	<u>2x</u>	<u>5x</u>	<u>16 ml:</u>	10.4 ml	6.8 ml	ddH <sub>2</sub> O	1.2 ml	2.0 ml	0.5M Tris pH 6.8	1.9 ml	3.2 ml	Glycerol	1.0 ml	1.6 ml	20%SDS	0.5 ml	0.8 ml	β-mercaptoethanol	1.0 ml	1.6 ml	1% Bromophenol blue	
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LB Media (Luria-Bertani)	1 liter in a 2-liter flask: Bacto-Tryptone 10 g Bacto-yeast extract 5 g NaCl 10 g ddH <sub>2</sub> O fill to 1 liter for plates: add 20g agar/liter in a 2-liter flask	<table border="1"> <tr> <td>Difco-Bacto 211705</td> </tr> <tr> <td>Difco-Bacto 212750</td> </tr> <tr> <td>Sigma (S-9625)</td> </tr> <tr> <td></td> </tr> <tr> <td>DifcoBacto 214030</td> </tr> </table>	Difco-Bacto 211705	Difco-Bacto 212750	Sigma (S-9625)		DifcoBacto 214030																
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Leupeptin 10mg/ml	10ml: 100 mg Leupeptin add DMSO to 10 ml Sterilize using a 0.22 μm filter. Store at -20°C.	Sigma (L-0649)																					
1M MgCl <sub>2</sub>	1 liter: 203.3 g MgCl <sub>2</sub> • 6H <sub>2</sub> O add ddH <sub>2</sub> O to ~800ml Adjust final volume to 1 liter with ddH <sub>2</sub> O. Aliquot and sterilize by autoclaving.	Sigma (M-9272)																					

4 M NaCl	1 liter: 233.76 g NaCl ddH <sub>2</sub> O to final 1 liter Aliquot and sterilize by autoclaving.	Sigma (S-9625)
3 M NaOAc	1 liter: 246.1 g NaOAc (Anhydrous) ddH <sub>2</sub> O ~750 mls adjust pH to 5.2 with ~140 ml of glacial acetic acid Adjust volume to 1 liter. Aliquot and sterilize.	Sigma (S-8750)
20X PBS	4 liters: 16.0 g KCl (53.4 mM KCl) 20.99 g KH <sub>2</sub> PO <sub>4</sub> • 3H <sub>2</sub> O (23.0 mM KH <sub>2</sub> PO <sub>4</sub> ) or 19.2 g KH <sub>2</sub> PO <sub>4</sub> 640.0 g NaCl (2.758 M NaCl) 172.87 g Na <sub>2</sub> HPO <sub>4</sub> • 7H <sub>2</sub> O (161.2mM Na <sub>2</sub> HPO <sub>4</sub> ) or 115.2 g Na <sub>2</sub> HPO <sub>4</sub>  Add ddH <sub>2</sub> O to ~3500 ml pH to 7.4 with NaOH pellets (~4.0 g) Bring final volume to 4 liters. Use vacuum filter top unit to remove any particles. Sterilize by autoclaving. Store at room temp.	Fisher (BP366-500) Sigma (P-0662)  Sigma (S-9625) Sigma (S-9390) Sigma (S-3397)
Pepstatin A 1 mg/ml	1ml: 1 mg Pepstatin A add methanol to 1ml. No need to filter sterilize. Aliquot and store at -20°C.	Sigma (P-4265)
Phenol:chloroform (25:24:1)	Phenol:Choroform: <i>iso</i> -amyl alcohol (25:24:1) 100ml: 50ml phenol (in buffered TE pH8.0) 48ml chloroform 2 ml <i>iso</i> -amyl alcohol mix well Store in a light-tight bottle at 4°C for 6 months  *Phenol is highly corrosive and can cause severe burns. Wear gloves when handling solutions containing phenol	Fisher (A92-112)

PMSF (10mM)	10ml: 17.4g PMSF Add isopropanol to 10ml and dissolve. No need to filter sterilize. Aliquot and store at $-20^{\circ}\text{C}$ . <i>PMSF is extremely unstable in aqueous solutions with a half-life of approximately 30 minutes and should be added immediately before use.</i>	Sigma (P-7626)
0.17M $\text{KH}_2\text{PO}_4$ , 0.72M $\text{K}_2\text{HPO}_4$	100ml: 2.31 g $\text{KH}_2\text{PO}_4$ monobasic anhydrous 12.54 g $\text{K}_2\text{HPO}_4$ dissolve in 90ml ddH <sub>2</sub> O. After the salts have dissolved, adjust the final volume to 100ml with ddH <sub>2</sub> O sterilize by autoclaving.	Sigma (P-0662) Sigma (P-3786)
Proteinase K (20mg/ml)	10ml: 200mg Proteinase K add dH <sub>2</sub> O to 10 ml Aliquot into 100-500 $\mu\text{l}$ and Store at $-20^{\circ}\text{C}$ .	Roche (745 723)
10X Reservoir Buffer (for SDS/PAGE)	2 liters: 60 g Tris base 288 g Glycine ddH <sub>2</sub> O to 2 liters add SDS to 0.1% final volume before use.	Sigma (T-1503) Sigma (G-7126)
RIPA Buffer	10mM Tris pH7.4 150mM NaCl 1% Sodium deoxycholate 1% Triton X-100 1mM PMSF 2 $\mu\text{g/ml}$ Leupeptin 2 $\mu\text{g/ml}$ Pepstatin  Store RIPA buffer at $4^{\circ}\text{C}$ . Ideally, the protease inhibitors should be added to the solution on the same day the assay is run but with the exception of PMSF the diluted inhibitors are stable in aqueous solution for up to 5 days.	100ml: 1M Tris pH 7.4 1ml 4M NaCl 3.75ml 10% Sodium deoxycholate 10ml 10 % Triton X-100 10 ml 10mM PMSF 10ml 10mg/ml Leupeptin 20 $\mu\text{l}$ 1 mg/ml Pepstatin A 200 $\mu\text{l}$ dH <sub>2</sub> O 65ml  Sigma (D-6750) Sigma (X-100)

RNAase that is DNAase-Free (10mg/ml)	10ml: 100mg RNAase A (pancreatic) dissolve in 10 ml of: 10mM Tris•Cl (pH 7.5), 15mM NaCl Boil 15 minutes. Allow to cool to room temp slowly. Dispense into aliquots and store at -20°C.	Sigma (R-5503)
4M NaCl	1 liter: NaCl add ddH <sub>2</sub> O to a final volume of 1 liter. Use vacuum filter-top to remove particles. Aliquot into 100ml bottles. Sterilize by autoclaving.	Sigma (S-9625)
10% SDS Sodium dodecyl sulfate	100 ml: (10%) 10g SDS add ddH <sub>2</sub> O to about 90ml Heat ~60°C to facilitate dissolving. There is no need to sterilize SDS.  Wear a mask while weighing SDS and wipe down area well as the fine crystals disperse easily. CAUTION: SDS is a strong denaturant and a major-league irritant.	BioRad (161-0302)
Semi Dry Transfer Buffer	5.82 g Tris 2.93 g Glycine 200 ml Methanol ddH <sub>2</sub> O to 1 liter Store in container with lid tight to prevent methanol from evaporating.	Sigma (T-1503) Sigma (G-7126)
20X SSC	1 liter: 175.3 g NaCl 88.2 g Citric acid (trisodium salt) add ddH <sub>2</sub> O to ~800ml pH to 7.0 with 12M HCl Adjust the volume to 1 liter with ddH <sub>2</sub> O pH w/~ 100 ml HCl	Sigma (S-9625) Sigma (C-7254)
20X SSPE	1 liter 174 g NaCl	Sigma (S-9625)

	27.6 g NaH <sub>2</sub> PO <sub>4</sub> • H <sub>2</sub> O monobasic 7.4 g EDTA adjust pH to 7.4 with ~6.5 ml 10N NaOH Sterilize by autoclaving.	Mallinkrodt 7892 Sigma (ED2SS)
50X TAE	200ml: 48.4 g Tris Base 11.42 ml Glacial Acetic Acid 20 ml 0.5 M EDTA (pH 8.0) add ddH <sub>2</sub> O to 200ml pH to 8.5 with NaOH pellets	Sigma (T-1503) Fisher (A38-212) Sigma (ED2SS)
5X TBE	1liter: 4liters: 54 g 216 g Tris 0.45 M Tris 27.5 g 110 g Boric Acid 0.5 M Boric Acid 20 ml 80 ml EDTA(pH 8.0) 0.5 M EDTA (pH 8.0)	Sigma (T-1503) Sigma (B-0252) Sigma (ED2SS)
TE 10mM Tris•Cl (pH8.0) 1mM EDTA (pH 8.0)	500 ml: 5ml 1M Tris (pH8.0) 1ml 0.5M EDTA (pH 8.0) 494 ml ddH <sub>2</sub> O Sterilize by autoclaving (if necessary).	
Terrific Broth	per liter: 12 g Bacto-tryptone 24 g Bacto yeast-extract 4ml glycerol add 900 ml ddH <sub>2</sub> O stir until the solutes have dissolved. sterilize by autoclaving. allow solution to cool to 60°C or less, then add: 100ml 0.17M KH <sub>2</sub> PO <sub>4</sub> , 0.72M K <sub>2</sub> HPO <sub>4</sub> (sterile)	Difco-Bacto 211705 Difco-Bacto 212750
Tetracycline 5mg/ml	20 ml: 0.1 g Tetracycline add ethanol to 20 ml wrap in foil in store at -20°C No need to filter sterilize. Store at -20°C. use at 10-50µg/ml	
1M Tris pH 7.4	1 liter: 121.1 g TrisBase	Sigma (T-1503)

	add ddH <sub>2</sub> O to ~800 ml Adjust pH to 7.4 with conc. HCl (~65 ml) Bring up volume to 1 liter	allow solution to cool to room temp before adjusting the final pH.
1M Tris pH 8.0	1 liter: 121.1 g Tris Base add ddH <sub>2</sub> O to ~800 ml Adjust pH to 8.0 with conc. HCl (~42 ml) Bring up volume to 1 liter	
1.5 M Tris pH 8.8	500ml: 90.825 g Tris Base add ddH <sub>2</sub> O to ~400 ml Adjust pH to 8.0 with conc. HCl Bring up volume to 500 ml	
0.5 M Tris pH 6.8	500ml: 30.275 g Tris Base add dH <sub>2</sub> O to ~400 ml Adjust pH to 6.8 with conc. HCl Bring up volume to 500 ml	