**Shared Reagents - Stone Chen Lab**

Sharing common reagents can significantly increase productivity. To enable us to do this, it is absolutely required that everyone in the lab be highly responsible and considerate in order to avoid issues including contamination of stock solutions, mistakes in making stock solutions, and abuse of reagents. Familiarize yourself with the preparation and use of the following reagents. Pay special attentions to <CAUTION>.

|  |  |
| --- | --- |
| Running buffer/medium  <CAUTION> For the aliquoted medium, once opening a new bottle, label and keep it only for your own use to avoid cross contamination. | |
| 10X SDS PAGE Buffer (1 L) | (0.25 M Tris, 1.92 M Glycine, 1% SDS)  30.3 g Tris  144.1 g Glycine  10 g SDS  1L with ddH2O  No need to adjust pH or filter. Store at RT.  **<CAUTION>** 1X SDS PAGE buffer can be reused > 5 times. |
| 10X TAE Buffer (1 L) | 48.5 g Tris  11.4 mL Acetic Acid Glacial  20 mL 0.5 M EDTA (pH8.0)  1L with ddH2O  No need to adjust pH or filter. Store at RT.  **<CAUTION>** To make 1X TAE buffer or agarose gel, add 2.5 uL of Ethidium Bromide per 100 mL solution.  **<CAUTION>** Do not re-use 1X TAE running buffer. Instead, collect the used buffer in the designated waste container. |
| 2X SDS Gel Sample Loading Buffer (50 mL) | 5 mL 1 M Tris pH 6.8  5 mL 2-mercaptoethanol  2 g SDS  0.1 g bromophenol blue  10 mL glycerol  50 mL with ddH2O  Aliquot 1 mL/tube, store at 4C |
| 6X SDS Gel Sample Loading Buffer  (50 mL) | 18 mL 1 M Tris-HCl pH 6.8  6 g SDS  30 g Glycerol  6 mg bromophenol blue  50 mL with ddH2O, microwave to dissolve. Store at RT.  **<CAUTION>** Before use, microwave to dissolve, take 1 mL and add 10 uL fresh BME. Immediately use it before it colds down. |
| 10X DNA Loading Buffer (50 mL) | 5 mL 1 M Tris-HCl pH 8.0  10 mL 0.5 M EDTA pH8.0  25 g Glycerol  0.15% Orange G  50 mL with ddH2O  Filter Sterilize, aliquot 1 mL/tube, store at 4C |
| LB for overnight culture (2 L) | Dissolve 50 g of LB powder in 2 L of DI water  Aliquot 500 mL/bottle, autoclave 30 min, store at 4C |
| NZY+ medium for transformation (1L) | 10 g NZ amine  5 g yeast extract  5 g NaCl  1 L with DI water, adjust pH to 7.5 using 10 N NaOH  Aliquot 250 mL/bottle, autoclave 30min, store at 4C  Before use, take 10 mL, add  100 uL 40% glucose  125 uL 1 M MgCl2  125 uL 1 M MgSO4  pre-warm in 42C water bath |
| SOC medium for transformation (1 L) | 20 g tryptone  5 g yeast extract  0.5 g NaCl  0.186 g KCl  0.952 g MgCl2  1 L with DI water, adjust pH to 7.4 using 10 N NaOH  Aliquot 250 mL/bottle, autoclave 30min, store at 4C  Before use, take 10 mL, add  100 uL 40% glucose  pre-warm in 42C water bath |
| LB agar plate (1 L) | 25 g LB powder  15 g Agar  1 L with DI water  Autoclave 30 min with a stir bar  Cool down with stirring for 40 min at RT  Add antibiotics, stirring for 5 min  Pour ~20 mL per plate near open flame  Color code the plates and leave on bench RT o.n.  The second day, wrap in plastic sleeves and store at 4C.  Color code:  Black: Ampicillin  Blue: Kanamycin  Green: Chloramphenicol |

|  |  |
| --- | --- |
| Supplements  Most of the supplements are very expensive. They are typically aliquoted and stored in -20C freezer. Do not put unfinished aliquots back into the lab stock. Instead, keep these to your own freezer box for your future use. | |
| 1000X Ampicillin (50 mL) | (100 mg/mL, Amp for short)  Dissolve 5 g Ampicillin in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 1 mL/tube, label each tube and store at -20C. |
| 1000X Kanamycin (50 mL) | (30 mg/mL, Kan for short)  Dissolve 1.5 g Kanamycin in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 1 mL/tube, label each tube and store at -20C. |
| 1000X Chloramphenicol (25 mL) | (34 mg/mL, Cm for short)  Dissolve 0.85 g Chloramphenicol in **Ethanol**, final volume 25 mL in conical tube.  Use parafilm to seal the tube and store at -20C. |
| 1000X Gentamicin (50 mL) | (20 mg/mL, Gen for short)  Dissolve 1 g Gentamicin in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 1 mL/tube, label each tube and store at -20C. |
| 1000X Tetracycline (10 mL) | (10 mg/mL, Tet for short)  Dissolve 0.1 g Tetracycline first in **10 mL of 95% Ethanol** in a 15-mL conical tube. The solution should be clear and bright yellow.  Filter sterilize. Use parafilm to seal the tube and store at -20C.  <**CAUTION**> Tetracycline is light sensitive. Wrap the Tet agar plates with foil for long term storage. |
| 1 M IPTG (50 mL) | Dissolve 11.9 g IPTG in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 1 mL/tube, label each tube and store at -20C. |
| 2000X Bluo-gal (1 mL) | (200 mg/mL, Invitrogen # 15519028, very expensive)  Dissolve 0.2 g in **100% DMSO**, final volume 1 mL. Store at -20C.  Solution is slightly blue when freshly made but change to bright yellow with time during storage. This does not affect usage. |
| 1 M DTT (50 mL) | Dissolve 7.7 g DTT in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 1 mL/tube, label each tube and store at -20C. |
| 1 M TCEP (25 mL) | (Mw 286.65)  Dissolve 7.17 g TCEP in 15 mL ddH2O  Adjust pH to ~7 using 10 N NaOH  Adjust final volume to 25 mL using ddH2O  Filter sterilize, aliquot 1 mL/tube, label each tube and store at -20C. |
| 0.1 M ATP (100 mL) | (Mw 551.14)  Dissolve 5.51 g ATP di-sodium in 100 mL of 100 mM Tris pH8.0  Filter sterilize, aliquot 4 mL/tube, label each tube and store at -20C. |
| 1000X Antipain (50 mL) | (1 mg/mL, Anti for short)  Dissolve 50 mg Antipain in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 0.25 mL/tube, label each tube and store at -20C. |
| 1000X Leupeptin (50 mL) | (1 mg/mL, Leu for short)  Dissolve 50 mg Leupeptin Hemisulfate in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 0.25 mL/tube, label each tube and store at -20C. |
| 1000X Benzamidine (50 mL) | (1 M, Benz for short)  Dissolve 7.83 g Benzamine in ddH2O, final volume 50 mL.  Filter sterilize, aliquot 0.25 mL/tube, label each tube and store at -20C. |
| 100X PMSF (50 mL) | (100 mM, **<CAUTION>** Toxic Chemical)  Dissolve 0.875 g PMSF in **isopropanol**, final volume 50 mL.  Aliquot 1 mL/tube, label each tube and store at -20C.  **<CAUTION>** PMSF is very unstable in water. Add it to cells right before lysis. To use, warm up an aliquot of PMSF in water bath, vortex until the crystals are fully dissolved. Do not put it on ice, which will crystallize PMSF. |

|  |  |
| --- | --- |
| Stock solutions  Filter sterilize the stock solutions to autoclaved glass bottles unless noted otherwise.  DO NOT directly pipet from the lab stock. Instead, transfer a desired volume to your own bottle, and pipet only from your own bottle. | |
| 1 M Tris-HCl pH8.5 or pH8.0 (2 L) | Dissolve 242.34 g Tris base in 1.6 L of ddH2O.  Adjust pH to 8.5 or 8.0 using concentrated HCl.  Transfer to a graduated cylinder, add ddH2O to 2 L final volume. Filter sterilize and store at RT.  **<CAUTION>** pH of Tris-HCl buffer is sensitive to temperature (~0.03 pH units for each 1C increase in temperature). Prepare the stock solution at RT. |
| 1 M HEPES pH 7 or pH7.5 (1 L) | Dissolve 238.3 g HEPES (free acid) in 600-700 mL of ddH2O.  Adjust pH to 7.0 or 7.5 using 10 N NaOH.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT. |
| 1 M Imidazole pH7 or pH8 (2 L) | Dissolve 136.15 g Imidazole in 1.6 L of ddH2O.  Adjust pH to 7.0 or 8.0 using concentrated HCl.  Transfer to a graduated cylinder, add ddH2O to 2 L final volume. Filter sterilize and store at RT.  Use pH7 for making KMEI buffers; use pH8 to make Ni beads elution buffers. |
| 0.5 M MES pH6 or pH6.5 (1 L) | Dissolve 97.6 g MES (free acid) in 800 mL of ddH2O.  Adjust to pH 6 or 6.5 with 10 N NaOH.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and **store at 4C, away from light**. |
| 0.5 M PIPES pH6.8 (0.25 L) | Dissolve 37.8 g PIPES (free acid) in 200 mL of ddH2O. Solution will be very turbid. Adjust to pH 6.8 with 10 N NaOH. Solution will become clear when pH is higher.  Transfer to a graduated cylinder, add ddH2O to 0.25 L final volume. Filter sterilize and **store at 4C, away from light**.  This buffer is typically only used for Arp2/3 complex purification. |
| 10X PBS (phosphate buffered saline) (1 L) | 2 g KCl  80 g NaCl  17.8 g Na2HPO4⋅2H2O  2.4 g KH2PO4  1 L with ddH2O  Filter sterilize and store at RT. |
| 3 M NaOAc (0.5 L) | Dissolve 204 g sodium acetate⋅3H2O in 400 mL of ddH2O.  Adjust to pH 5.2 with glacial acetic acid.  Transfer to a graduated cylinder, add ddH2O to 0.5 L final volume. Filter sterilize and store at RT. |
| 5 M NaCl (2 L) | Dissolve 584.4 g NaCl in EXACTLY 1760 mL ddH2O. Stir until the salt is fully dissolved.  Transfer to a graduated cylinder, add ddH2O to 2 L final volume. Filter sterilize and store at RT.  **<CAUTION>** 5 M is close to saturation point of NaCl. It does not fully dissolve until the final volume is very close to 2 L. |
| 3.5 M KCl (2 L) | Dissolve 512.9 g KCl in 1600 mL ddH2O. Stir until the salt is fully dissolved.  Transfer to a graduated cylinder, add ddH2O to 2 L final volume. Filter sterilize and store at RT.  **<CAUTION>** 3.5 M is close to saturation point of KCl. It does not fully dissolve until the final volume is very close to 2 L. |
| 1 M MgCl2 (1 L) | Dissolve 203.3 g of MgCl2⋅6H2O in 800 mL of ddH2O.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT.  **<CAUTION>** MgCl2 is highly hygroscopic. Seal the bottle using parafilm after use. |
| 1 M MgSO4 (1 L) | Dissolve 246 g of MgSO4⋅7H2O in 800 mL of ddH2O.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT. |
| 1 M NiSO4 (1 L) | Dissolve 262.8 g of NiSO4⋅6H2O in 800 mL of ddH2O.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT.  **<CAUTION>** This is a toxic metal. The diluted solution needs to be collected in the designated waste container. |
| 0.5 M EDTA pH8 (1 L) | Dissolve 186.1 g Na2•EDTA•2H2O in 800 mL of ddH2O  Add 18 g NaOH, and stir until all the NaOH dissolves.  Adjust to pH8.0 with 10 N NaOH  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT.  **<CAUTION>** EDTA will not completely dissolve until pH8. |
| 0.5 M EGTA pH8 (200 mL) | (Mw 380)  Dissolve 38 g EGTA in 180 mL of ddH2O  Add 3.5 g NaOH, and stir until all the NaOH dissolves  Adjust to pH8.0 with 10 N NaOH  Transfer to a graduated cylinder, add ddH2O to 200 mL final volume. Filter sterilize and store at RT.  **<CAUTION>** EGTA will not completely dissolve until pH8 |
| 10 N NaOH (500 mL) | Dissolve 200 g NaOH in a final volume of 500 mL ddH2O  Filter through a PES filter, and store at RT.  **<CAUTION>** Use NaOH-compatible filters (e.g. PES, but not SFCA) |
| 40% Glucose (1 L) | Add 500 mL of ddH2O to a 1-liter beaker.  With continuous stirring, add 400 g of glucose (dextrose) to the beaker until fully dissolved.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT.  **<CAUTION>** You would make a huge candy bar if adding water to glucose powder instead. |
| 20% Maltose (1 L) | Add 500 mL of ddH2O to a 1-liter beaker.  With continuous stirring, add 200 g of maltose to the beaker until it is fully dissolved.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter sterilize and store at RT. |
| 10% L-Arabinose (0.25 L) | Add 150 mL of ddH2O to a 1-liter beaker.  With continuous stirring, add 25 g of maltose to the beaker until it is fully dissolved.  Transfer to a graduated cylinder, add ddH2O to 250 mL final volume. Filter sterilize and store at RT. |
| 80% (w/v) glycerol (1 L) | Weigh 800 g of glycerol in a 1-liter glass bottle.  Add water to 1 L.  Manually mix it till the solution is homogenous.  Add water to 1 L final volume  Manually mix it again till the solution is homogenous. Keep at RT. |
| 10% (w/v) SDS (1 L) | Dissolve 100 g of SDS in 800 mL of ddH2O. Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Store at RT.  **<CAUTION>** Wear a mask when weighing SDS and wipe down the weighing area and balance after use because SDS is very light. |
| 10% (w/v) NP40 (1 L) | (also named IGEPAL)  Pour 100 g NP40 to a 1-liter beaker containing 800 mL of ddH2O, stir until it is fully dissolved. This will take ~ 1 hr.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Store at RT. |
| 10% (w/v) Triton X-100 (250 mL) | Pour 25 g Triton X-100 to a beaker containing 200 mL of ddH2O, stir until it is fully dissolved. This will take ~ 1 hr.  Transfer to a 250-mL graduated cylinder, add ddH2O to 250 mL final volume. Store at RT. |
| 10% (w/v) Tween20 (250 mL) | Pour 25 g Tween20 to a beaker containing 200 mL of ddH2O, stir until it is fully dissolved. This will take ~ 1 hr.  Transfer to a graduated cylinder, add ddH2O to 250 mL final volume. Store at RT. |
| 6 M Guanidine HCl (1 L, for cleaning beads) | (Mw 95.53)  Weigh 573 g GuHCl in a 1 L beaker  Add ddH2O to dissolve to ~ 900 mL  Cover the beaker with plastic wrap, and microwave the solution ~3 minutes. **<CAUTION>** Bottom of the beaker may be hot now.  Stir until all salt dissolves. If not, microwave again.  Transfer to a graduated cylinder, add ddH2O to 1 L final volume. Filter through 0.45um membrane and Store at RT.  **<CAUTION>** For cleaning Ni-NTA beads, use 6 M GuHCl supplemented with 0.2 M (~ 1.2%) acetic acid. |