

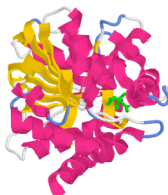
## Biochemistry Lab II

### List and location of Reagents



### Stock Solutions, Media, Reagents and Materials

Item	Concentration / Volume	Location
Ampicillin	Stock concentration is either 500X (50 mg/ml) or 1000X (100 mg/ml) in H <sub>2</sub> O. Final concentration = 100-200 µg/ml Do not add until the media is cool to the touch!	Microfuge tubes in plastic storage boxes in the freezer <b>- Re-freeze unused reagent</b>
Chloramphenicol	Stock concentration is either 200X (34 mg/ml) in ethanol. Final concentration = 170 µg/ml Do not add until the media is cool to the touch!	Microfuge tubes in plastic storage boxes in the freezer <b>- Re-freeze unused reagent.</b>
Kanamycin	Stock concentration- 500X (25 mg/ml) in H <sub>2</sub> O. Final concentration = 25 µg/ml. Do not add until the media is cool to the touch!	Microfuge tubes in plastic storage boxes in the freezer <b>- Re-freeze unused reagent</b>
LB Medium	10 g/L tryptone, 5 g/L yeast extract, 10 g/l NaCl. <b>OR</b> use the pre-mixed powder as directed. <i>Autoclave, but do not fill more than 80% of capacity.</i> For 1 liter cultures, use the 2 liter flasks. <b>LB media (w/o antibiotics) is in 50 ml aliquots in falcon tubes in fridge.</b>	Individual items are in the cold room. Pre-mixed powder in front cabinet of lab.
LB Agar	LB medium containing 15 g of Agar <b>OR</b> use pre-mixed powder as described on the bottle.  IMPORTANT: Agar is solid when prepared. Microwave until clear. Cloudy agar is not completely molten and will be "loose" when cooled.  <b>LB plates with your colonies will be in the refrigerator. DO NOT FREEZE these plates.</b>	Individual items are in the cold room. Pre-mixed powder in front cabinet of lab.
Culture Plates	Large plates (100 mm) need 10 - 15 ml per dish Medium Culture plates (60 mm) use about 10 ml (1/2 filled) per dish	Placed in front cabinet of room Stocks in cabinet under sink
IPTG	Stock concentration is 1M (238 mg/ml in H <sub>2</sub> O). Final conc. Should be 1 mM. ~ 0.5 ml aliquots	Stored in expression kit in freezer. Each group will check out kit. <b>Re-freeze when done!</b>
PBS	10x Stock Solution pH 7.2 <ul style="list-style-type: none"> <li>o 80 g NaCl</li> <li>o 2 g KCl</li> <li>o 11.5 g Na<sub>2</sub>HPO<sub>4</sub> H<sub>2</sub>O</li> <li>o 2 g KH<sub>2</sub>PO<sub>4</sub></li> </ul>	Front Cabinet - Premade 10X & 1X PBS is prepared in lab. Use MilliQ Water to dilute. Only make what you need.
Tris-HCl	Trizma and Tris-HCl are one and the same. Tris base is the base form and will need to be pH adjusted differently	1 M stock is stored in front of room. pH as needed.
EDTA	Stock solution is 0.5 M Tetrasodium EDTA Salt. Free acid is difficult to dissolve without adjusting pH.	500 mM EDTA is stored in cabinet in front of room
PMSF	Stock concentration = 200 mM in Ethanol.	Stock in cabinet in front of room
20% Triton X-100	Dissolve 10 ml Triton X-100 into 50 ml total H <sub>2</sub> O. It may take 1 or more hours rocking to dissolve. Store in glass. Concentrated (100%) is very viscous. Use care when measuring.	Stock and concentrated detergent in glass bottle in cabinet in front of room.



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MSUM Biochemistry

5X SDS Page Sample buffer	See SDS-PAGE gel instructions. NOT USED FOR DNA. Stored in 1 ml falcon tubes.	In front cabinet. Return unused sample buffer!
Sonnicator	Be careful to follow directions and clean and dry when done. Use the ear-protection when using. NEVER leave the sonnicator on without being in the solution or touch the tip to the side. This includes turning the power down. It must be off when not in use.	Dark Room READ INSTRUCTIONS!  Do not use without specific instructions from instructor.
Needles syringes	Place needles in sharps container when finished. Wash and replace the barrels back container next to pH meter.	
Plastic Cuvettes	Blank with media only. It is best to use a transfer pipet to save some for the duration of the experiment.	
Falcon Tubes	Wash and replace when done. If stress fractures appear, discard. Re-used tubes are fine for many purposes.	Each group has two in drawer. Wash and re-use. Do not throw away.
Ethidium Bromide	10 ng/ml stock. Transfer all solutions and agarose containing EtBr to large container in hood in Hagen 102.	
DNA Loading Soln		
Agarose	0.8 or 1% is typically enough. Dissolve in TEA or TBE, not water. Add 40 $\mu$ l Ethidium Bromide (10 ng/ml) to running buffer before preparing gel and using in electrophoresis.	
TAE	50X solution <ul style="list-style-type: none"> <li>o 242 g Tris base</li> <li>o 57.1 ml glacial acetic acid</li> <li>o 37.2 g Na<sub>2</sub>EDTA·2H<sub>2</sub>O</li> <li>o QS to 1 liter with H<sub>2</sub>O</li> </ul>	
Centrifuge Tubes	500 ml tubes need to be washed and returned ASAP. Do NOT use centrifuge without prior clearance from Dr Provost	
Imidazole	Use your solutions within a few weeks. Store the solutions you make in the cold room. Be certain to label with your name and the contents of the bottle. Not just elution buffer.	1 M Imidazole - Frozen aliquots to be re-used when done.
Ni-Columns.	Use the slurry in the labeled bottle (For Biochem) ONLY. When you are done, transfer it into the bottle labeled used Ni-Agarose. It is expensive and can be re-used after regeneration.	Will be checked out to you.
Bradford Protein Reagent	The 1X solution is stored in a labeled bottle. Same as protein assay. A 5X solution is purchased from BioRad.	
MDH assay	OAA, NADH (DNPH), and other materials are solids in the freezer. OAA can be prepared and saved as a frozed solution. NADH must be made up fresh each time. For other materials ask JP	Freezer