Standard Laemmli Gel Solutions

U. K. Laemmli (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* 227, 680 – 685.
(For many years, this was the most-cited paper in all of scientific research b/c of the thousands of applications).

NOTE: Acrylamide is a known neural toxin that acts on the intermediate filament systems of cells; its action is fast and it causes long term damage. Acrylamides are suspected carcinogens. Wear gloves, be very careful. SeeMSDS:
http://www.jtbaker.com/msds/englishhtml/A1550.htm

Sol. A: 30% Acrylamide:bis-acrylamide at 37.5:1.
Liquid stock of 37.5:1 stock from Fisher (BP1406) is supplied at 40%:
Dilute small quantities of 40% stock to 30%.

Ultrapure water	33 mL
40% Fisher liquid stock	100 mL

Shelf life of unperturbed Fisher stock solution is years, although the manufacturer indicates only ~ 6 months.

Alternatively, make from dry ingredients (*much more hazardous*). IN A HOOD, make:
Acrylamide:bisacrylamide 30:0.8. (30:0.8 = 37.5:1 & works identically.)

Acrylamide	300g
N' N' Bisacrylamide.	8g
Ultrapure water	*Bring to* 1.0 L

Keep all acrylamide solutions refrigerated and in a brown or foil-wrapped bottle.

Sol. B: 1.5 M Tris buffer, pH 8.8. This is the separating gel buffer. Alas, Tris is also not good for you (but there are worse things). See MSDS:
http://www.jtbaker.com/msds/englishhtml/t7112.htm

Tris base (FW=121.1)	18.15 g	90.75 g	181.5 g
Ultrapure water *final volume*	100 mL	500 mL	1 L

Adjust to pH 8.8 with 1.0 M NaOH

Solution C: 20% SDS. Used to maintain protein denaturation. *Caution! SDS is an ionic detergent, and can wreak havoc with delicate skin and your lungs! Always weight out to minimize dust, or weight in a hood (difficult to do). See MSDS:*
http://www.jtbaker.com/msds/englishhtml/s3670.htm

Sodium dodecyl sulfate (SDS)	20g
Ultrapure water *final volume*	100 mL

As with all reagents, the chemical properties are not precisely the same for all manufacturers, and researchers have taken advantage of this for many years. In order to get optimal separation of tubulin and dynein heavy chains from sea urchin, use crude Sigma - "sodium lauryl sulfate" #5750. For optimal separation of Chlamydomonas and fish (trout and striped bass) dyneins, use highly purified Fisher # BP166-100.

Solution D: 0.5 M Tris, pH 6.8. This is stacking gel buffer.

Tris base (FW=121.1)	3g
Type I water. *Final volume*	50mL

Laemmli sample buffer (LSB). We make a very concentrated version and dilute with the sample. The advantage of this approach is that if the sample is not very concentrated then we get more total protein into the final gel sample. We 'fudge' a 5X solution, which literally cannot be made to the original composition; this solution has proven to be very workable. The 4X solution that follows is literal to the original composition.

5X LSB:

20% SDS	3 mL
glycerol	3 mL l00% stock
Tris	0.454 g

Bring the solution to pH 6.8, using either a designated pH electrode or pH strips.

Add a little Bromophenol Blue to make solution very dark. The Bromophenol Blue can be left out and added immediately before use. This method has the advantage that the LSB can be kept on the shelf for years without breakdown of the dye. Dye breakdown is not a critical problem, but causes two distinct dye fronts in 5-15% gradient gels. Dye breakdown is evident from the change in color of the solution; it goes from a Navy-blue to a more purplish color.

Bromophenol blue is not wicked, but like everything else here, needs to be handled with respect: MSDS: *http://www.jtbaker.com/msds/englishhtml/b5056.htm*
Glycerol (=glycerine) is a component of soaps, shampoos and skin care lotions. Then again, so is SDS! MSDS: *http://www.jtbaker.com/msds/englishhtml/G4774.htm*

4X LSB:

Tris HCI (pH 6.8) SDS	1.52 g	0.25M
SDS	4.0 g	8 %
Glycerol	20 mL	40 %
Ultrapure water, bring to	50 mL or what ever is needed to accommodate the mercaptoethanol see below:	

Mercaptoethanol (Fisher #BP176-100) is added just before use as described above.
Excessive use of mercaptoethanol can cause cross-linking reactions between the protein and acrylamide, causing smearing in the gel. Although the original recipe calls for 20% final mercaptoethanol, we keep the total mercapoethanol concentration below 10 % in the stock. 1% final in the solution is probably plenty for most protein preparations.

Bromophenol Blue dye breakdown (see above) is greatly hastened by addition of 2-mercaptoethanol, so we usually leave that out until immediately before the procedure. The additional advantage of adding mercaptoethanol late is that this approach allows one to test for the presence or absence of disulfide bonds in the protein (do runs with/without the mercaptoethanol; gel runs without mercaptoethanol make the protein appear smaller than with the mercaptoethanol). *Mercaptoethanol* is very smelly; it is also hazardous; minimize exposure; use in a hood wherever possible. However, it is unrealistic that you use mercaptoethanol in a hood all the time. Gels are run on the bench. MSDS: *http://www.jtbaker.com/msds/englishhtml/m1209.htm*