
SECTION I

EUKARYOTIC DNA

MAKING SOLUTIONS

In most courses the TA or instructor hands you all of the solutions that you need for the experiments. This course will be different, in that you or members of your group will make some of your own solutions. The exceptions will be when hazardous or expensive solutions need to be made or when the solutions require long times for preparation. These solutions will be provided to you. The reasons for this exercise are: 1) to get you acquainted with the lab and others in your group, 2) to get you started thinking about some of the measurements involved in molecular biology, and 3) to test your skill with solutions.

In molecular biology, most solutions are in the millimolar (mM) ranges. Many organisms can easily live in these solutions. Because of this most are first made as stock solutions which are of higher concentrations, so that microorganism contamination is less of a concern. Also, many of the solutions will be autoclaved or filter sterilized to exclude possible contamination.

As you remember from basic chemistry, molarity is defined as the concentration of a solute (based on its molecular weight) dissolved in a solvent (for our use this is almost always water). A 1 molar (1M) solution is one molecular weight of the solute dissolved in a final volume of 1 liter of the solvent. Remember as you make these solutions your success in the following exercises will depend on making the solutions of the correct constituents and molarities. Take care with your calculations and when weighing out the chemicals. Also, make only enough to be used for this course. Do not make excessive amounts, since this will be a waste of chemicals. As with pipette tips, never put a used spatula into a container of a chemical. Always rinse and dry the spatula first. Also, never return the chemical back into the container. Just throw the excess out. Otherwise the chemical may become contaminated

HERE ARE A FEW EXAMPLES:

1. To make 1 liter of a 1M solution of NaCl (mol wt = 58.44 g/mole), 58.44 g of NaCl is dissolved in water, the final volume being 1 liter.
2. To make 100 ml of a 5M NaCl solution, 5 moles/liter \times 58.44 g/mole \times 0.1 liter = 29.22 g of NaCl is dissolved in water to a total volume of 100 ml.
3. For acids and bases, normality is often used as the measured concentration. Normality is a more direct measure of the ion concentration of the solution, and therefore for acids and bases is more indicative of the pH of the solution. For strong acids, such as NaOH and HCl, normality and molarity are almost identical because in solution nearly all of the ions are dissociated from one another. [For weak acids and bases, only a proportion of the ions are dissociated from one another.] To make 1 liter of a 1 normal (1N) solution of NaOH (mol wt = 40.00), 40.00 g of NaOH is dissolved in

water to a final volume of 1 liter.

4. To make a 1N solution of HCl you have to know the concentration of the concentrated HCl that comes in the bottles from the factory, which is about 12N. To make 1 liter of a 1N solution, 1/12 of the final volume (1 liter) should be HCl, therefore 83.33 ml of concentrated HCl would be dissolved in water to a total volume of 1 liter.

5. To make millimolar concentrations generally a more concentrated stock solution is first made and then a portion of this is diluted to achieve the final concentration. TE (Tris-EDTA) is 10 mM Tris (pH 8.0) and 1 mM EDTA. Rather than measuring out small amounts in order to make 100 ml of TE, a 1M Tris solution and a 0.5M EDTA solution are made and diluted into the final solution. Therefore, 10mM/1000mM or 1/100 or simply 1 ml in every 100 ml (total) of 1M Tris is added to the solution, and 1mM/500mM or 1/500 or simply 200 μ l per 100 ml of 0.5M EDTA is added to the solution. To make 50 ml of TE, 500 μ l (0.5 ml) of 1M Tris and 100 μ l (0.1 ml) of 0.5M EDTA is added to water to a final volume of 50 ml.

6. Some solutions are described in terms of percent rather than molarity or normality (for various reasons). Two forms of measurement are used: weight by percent (w/w, which isn't used very often) and weight-volume percent (w/v, which you will see fairly often). A 1% (w/w) solution is 1 g of the chemical plus 99 ml of the solvent. On the other hand, a 1% (w/v) solution is 1 g of the chemical dissolved in the solvent to a total volume of 100 ml. Therefore, to make 100 ml of a 2% (w/v) sucrose solution, 2 g of sucrose is dissolved in water to a final volume of 100 ml.

If you have any problems making any of the solutions or calculating amounts, please ask the TA or the instructor for assistance.

Make the following solutions (per group):

1. 100 ml 80% EtOH [Dilute 95% EtOH.]
2. 250 ml 5X TBE [Use powdered Tris Base and Boric Acid, and the 0.5 M solution of EDTA.]
3. 25 ml 1X TE (pH 8.0) [Dilute 10X TE.]
4. 1 ml 0.1X TE (pH 8.0) [Dilute 1X TE.]
5. 50 ml 2X CTAB buffer [Use powdered CTAB, NaCl and PVP, and solutions of Tris and EDTA.]
6. 100 ml CTAB precipitation buffer [Use powdered CTAB, and solutions of Tris and EDTA.]
7. 50 ml High-salt TE buffer [Use solutions of all three constituents.]

[NOTE: You will find the recipes for these solutions in the "DNA extraction" and "Gel electrophoresis" procedures.]

You should also autoclave some microfuge tubes:

1. Pour some 1.5 ml microfuge tubes into a 500 ml beaker and place two layers of aluminum foil over the top, then autoclave for 20 minutes, with an added 10 minutes on the dry cycle.
2. Do the same for 0.5 ml microfuge tubes in a 250 ml beaker.