Aqueous Solutions (*modified from Maureen Munn at UW Genome Sciences)*

Have you ever wondered why DNA and protein solutions contain so many ingredients? Most of these components are added to stabilize the native structure of the protein or DNA during and after purification. The inside of a cell contains a huge variety of proteins, amino acids, salts, sugars, fats, etc., so that the cytosol is extremely viscous—about the consistency of egg white. Also, the cells of eukaryotic organisms (i.e., animals and plants) are subdivided into different organelles, such as the nucleus, the Golgi bodies, mitochondria, and lysosomes, and inside each organelle the environment is specialized.

When we grind or blend cells at the beginning of a purification, these delicate organelles are disrupted and their contents are mixed. Molecules such as nucleases (these cut up DNA and RNA) and proteases (these cut proteins), which are normally sequestered in organelles, can come in contact with the molecules that we are purifying. There can also be dramatic changes in the pH of the solution. Many reagents are added during purification to counteract these problems. After purification there is usually a single biological molecule in the sample. This can be damaging to a complex biological molecule because it is no longer in contact with other biological molecules that would normally stabilize it in the cell. So, we add a number of different ingredients to take the place of all the cellular components that were purified away. Some of these ingredients are discussed below.

1. **Tris-HCl** acts as a buffer in an aqueous solution over the pH range from 7.0-9.0. Thus, in this biologically useful pH range, it helps to maintain a constant pH when small amounts of acids or bases are added. DNA is an acid, so if it is stored in an unbuffered solution, it can cause its own destruction through acid hydrolysis. Proteins are also very sensitive to pH, and an enzyme may not function outside its narrow pH optimum even though its structure does not appear to be altered.

2. Salts such as **NaCl** or **KCl** help to adjust the ionic strength of the solution to be equivalent to that inside the cell. One important role of these ions is to interact with the charged groups on the surface of proteins and DNA. Divalent cations, especially Mg^{++}, are often required by DNA-modifying enzymes for their enzymatic activity.

3. **Glycerol** is a viscous organic liquid that mixes with water. It has three special properties that make it useful in solutions of biological molecules: 1) it binds water molecules; 2) it is denser than water; and 3) its freezing point is much lower than that of water. Glycerol is often added to protein solutions during and after purification to bind some of the water molecules in the solution and make them less interactive. This helps mimic the environment inside a cell, where much of the water is bound to other protein molecules.

   Because of the low freezing point of glycerol, many enzymes are stored in 50% glycerol so that they remain unfrozen at -20°C. This has a dual advantage—a small aliquot of the enzyme can be removed from the storage tube without the necessity of thawing or even warming the enzyme, and we avoid potential damage to the enzyme that would be caused by the formation of ice crystals. Glycerol is often added to sample loading

buffers to make the samples denser than the gel running buffer, and that helps the sample to settle into the well of the gel during loading.

4. **Distilled water** is used in all solutions containing biological molecules because tap water contains trace amounts of salts, heavy metals, chlorine, fluorine, and dirt that can affect the biological molecules under study. The trace amounts of heavy metals that are generally present in tap water can damage DNA and inhibit some enzymes.

5. **EDTA** is a chemical that chelates (i.e. binds noncovalently) to heavy metals like Zn^{++} and to divalent cations such as Mg^{++}. EDTA is added at low concentrations to inactivate any trace heavy metals that might otherwise inhibit an enzymatic reaction. Because it binds Mg^{++}, it is also used to stop enzymatic reactions that require Mg^{++}. For example, the restriction enzyme buffers used for digesting DNA with BamHI, EcoRI, and Hind III contain 10 mM $MgCl_2$. At the end of the incubation period, we add 3 µl sample loading buffer, which contains 50mM EDTA. This is a slight molar excess over the amount of Mg^{++} in the reaction, so EDTA's action inhibits the restriction digestion.

6. **TAE** is tris-acetate-EDTA which is a running buffer used for running and separating large pieces of DNA. It is typically used for running gels at low voltages, i.e. <150V.

7. **TBE** is tris-borate-EDTA which is a running buffer used for running and separating small pieces of DNA. Using TBE gives higher resolution of small DNA fragments (0.1 to 3kb). TBE also has higher buffering capacity and lower conductivity than TAE and therefore is typically used when running gels at high voltages, i.e. 2000V. Either TAE or TBE can be used in agarose gel electrophoresis using the range in sizes of DNA pieces used in SEP's DNA Labs 1 & 2.

8. **SB** is sodium boric acid, which is a conductive running solution that provides ions necessary for gel electrophoresis of proteins and RNA. Current research indicates that SB is an alternative to tris-based buffers used in DNA gel electrophoresis. SB allows DNA gels to be run at higher voltages for shorter times without melting the gel or denaturing the DNA.

Slab and Flat Bed Gel Electrophoresis, Agar, and Agarose

Gel electrophoresis is a technique that is used to separate several kinds of molecules. The gel itself is actually a sieve, which serves as a solid matrix in which to contain the molecules during separation. Depending on the size of the molecules being separated, the appropriate sieve may be a high or low percentage agarose, or a fixed or gradient percentage polyacrylamide. The process of gel electrophoresis entails running a DC current from a power supply to one electrode in a buffer chamber, through a gel (either horizontal (flat bed) or vertical (slab)), and into the other buffer chamber to the other electrode and back to the power supply to complete the circuit. In some gel boxes, the only electrical connection between the two chambers is the gel; electric current passes through the gel. In the horizontal gel box that SEP uses (Horizon™ 58), the gel sits submerged in running buffer, which is also in the chambers on opposite ends of the gel bed. Once the sample molecules are loaded in the wells of the gel and voltage is applied, the molecules enter the gel matrix, and migrate in response the electric field. Their rate of migration through the electric field depends on the net charge, size and the shape of the molecules; and, also on the ionic strength, viscosity, and temperature of the medium in which the molecules are moving.

To improve separation of bands or to improve resolution of bands, the type of running buffer and the amount of voltage are considerations. For SEP's DNA labs, we use agarose gels. In agarose gels, the pore size of the sieve can be decreased by increasing the percentage of the agarose in the gel. The amount of agarose in gels can range from 0.3% to 4% w/v.

8. **Agar** is a phycocolloid extracted from carrageen red algae (seaweed). The main components of agar are agarose and agaropectin. It has a low gelling temperature and a high melting point, which makes it an ideal solidifying agent. It is the preferred medium for growing bacteria because most microorganisms can't metabolize the polysaccharide galactopyranose, which is the component of agarose. Agar cannot be used for gel electrophoresis because it is contaminated with other charged materials that would interfere with the gel running process.

9. **Agarose** is purified agar, i.e. only the agarose portion, which is made up of a polymer comprised of repeating molecules of two forms of galactopyranose. It is ideal for gel electrophoresis because agar has no net charge and therefore will not interfere with the running buffers being utilized or the molecules being analyzed in the gel running process.