Making Solutions

Perhaps you've made solutions for chemistry. Generally these solutions combine percent solutions of a single compound with percent solutions of another compound in given ratios. With biological samples (living) such as tissues, cells, or fractionated cells, the requirements for bathing solutions/buffers is often quite different.

There are several factors that must be met to keep the components of a biological system viable. For whole cells or organelles, the medium must have a particular osmolarity in order to minimize swelling and shrinking of the cells/organelles. The common goal for cell/organelle suspension solutions is an osmolarity of around 250 mM. This represents the composite of all dissociated and dissolved compounds within the cytosol of the cell. It is also the osmolarity of a "physiologic saline" solution that might be given to a patient in the hospital after surgery, digestive problems, or blood loss.

The second factor that is true for experiments using living organisms/cells/organelles is that they require a buffered environment. This means that they need a compound within their suspension solution that can minimize pH changes. These types of compounds are called buffers. Some commonly utilized buffers are Tris, Sodium phosphate, MOPS and HEPES. Buffers are selected for the range of pHs that they can buffer, but most of those used in biological research have buffering around pH 7.

Other factors that are common to many physiological solutions are the inclusion of sucrose as an osmolarity regulator (rather than, or in addition to salt), or inclusion of other compounds vital to the survival of the cell/organelle/organism of interest.

Lastly, when we take cells/organelles/organisms out of their regular environment, they tend to degrade rather quickly. This is why physiologic solutions are typically kept on ice. Because of the presence of organic compounds (such as sugar), these solutions are also prone to bacterial contamination, so they should be stored in the refrigerator, or in some instances, in the -20^{o}C freezer.

<u>Preparing mg/ml solutions</u>

Preparing Percentage Solutions

Sometimes you will be called upon to prepare a solution that is expressed as a percentage. This is by far the simplest solution to make. An X% solution contains X g/ 100 ml of solution.

For example, if you need to make 60 ml of a 5% solution of pyruvate you would use the following expression to calculate the amount of pyruvate:

x g/60 ml = 5 g/100 ml = 3g

You would add 3 g of pyruvate to less than 60 ml of water.

When the pyruvate is dissolved, you would adjust the final volume to 60 ml.

Storing Solutions

It is common practice to NEVER store solutions going into the freezer in glass containers. Find a plastic container or tube (not a screw-top tube) that is of appropriate volume. Label with specific contents (ie. 100 mM Tris, pH 8—NOT CS assay buffer), your initials, and the date.

Temperatures for storage are as follows:

4-8^{o}C is refrigeration. Glass containers with lids can be used in a fridge. It is poor practice to put parafilm on a flask and keep it in the fridge. There is too much of a possibility of spillage.

-20^{o}C is a common freezer. Some items may need to be in a non frost-free environment. Newer fridge/freezers actually defrost the freezer on a regular basis, resulting in pretty big fluctuations in temperature, whereas chest freezers do not auto-defrost. You should always keep items such as restriction enzymes and PCR materials in a non frost-free environment Other items that need to be frozen, but are not quite so sensitive to temperature fluctuations, can be kept in a freezer portion of a fridge/freezer combo. Never use glass for storage containers.

-80^{o}C is an ultra-cold freezer. Samples that can lose activity over time are generally stored in this type of freezer so that it can prevent degradation or loss of activity. Items stored in this freezer should be in a freezer box marked with your name and date. Typically, samples kept in this type of freezer are small, and are NEVER stored in glass containers.