

## Andrea's Help Sheet on Preparing Solutions

There are several types of stock solutions made in the research lab: Percent (%) solutions, Molar (M) solutions, X solutions, and mg/ml solutions. First are instructions on how to make % solutions.

### First, know the definition of a % solution: $1\% = 1\text{g}/100\text{ml}$

That's the basic formula, and it is logical because "per cent" means "per hundred".

So, let's say you are assigned the task of making 1 liter of 20% SDS stock solution, a lab basic. (On quizzes, you may be told the MW of the chemical; it is not used in % solutions, so ignore it.)

### The three steps are:

- 1: write out the formula:  $1\% = 1\text{g}/100\text{ml}$
- 2: scale it up on the percentage:  $20\% = 20\text{g}/100\text{ml}$
- 3: scale to the final volume by multiplying by 10:  $20\% = 200\text{g}/1000\text{ml}$

Write out the description in the following manner:

*Place 200g of SDS powder a 1L beaker and add H<sub>2</sub>O up to 900ml. When dissolved, transfer solution to a 1L graduated cylinder and add H<sub>2</sub>O to 1000ml.*

This works whether for a % w/v solution (as exempld above) or a % v/v solution. Suppose you need 100ml of 20% EtOH:

$$\begin{aligned}1\% &= 1\text{ml}/100\text{ml} \\20\% &= 20\text{ml}/100\text{ml}\end{aligned}$$

*Mix 20ml EtOH with 80ml H<sub>2</sub>O for a final volume of 100ml.*

Next, let's look at Molar (M) solutions.

### First, know the definition of a Molar solution: $1\text{M} = 1\text{MW}(\text{in grams})/\text{Liter}$

(MW = molecular wt.) That's the basic formula, and is logical because Molar is defined as a solution concentration having one mole of solute per liter of solution.

So, lets say you are assigned the task of making 100ml of 5M NaCl. Here, you **will** need the molecular wt for NaCl; it is 58.44.

### The three steps are:

- 1: write out the formula:  $1\text{M} = 1\text{MW}(\text{g})/1000\text{ml}$
- 2: plug in the numbers & scale it up on the Molarity:  $5\text{M} = 5(58.44)\text{g}/1000\text{ml}$   
& work out the calculations:  $5\text{M} = 292.2\text{g}/1000\text{ml}$
- 3: scale to the final volume by dividing by 10:  $5\text{M} = 29.22\text{g}/100\text{ml}$

Write out the description in the following manner:

*Dissolve 29.22g of NaCl in 90ml H<sub>2</sub>O in a 100ml beaker. Transfer solution to a 100ml graduated cylinder and add H<sub>2</sub>O to 100ml.*

If you are assigned a specific pH, make sure to titrate the solution in your description:

Making 500ml of 1M Tris, pH 8.2 would go like this (Tris MW = 121.14):

$$\begin{aligned}1\text{M} &= 121.14\text{g}/1000\text{ml} \\1\text{M} &= 60.57\text{g}/500\text{ml}\end{aligned}$$

*Dissolve 60.57g of Tris in 450ml H<sub>2</sub>O in a 500ml beaker. Titrate the solution to 8.2 using either HCl (acid) or NaOH (base) as needed. Transfer the solution to a 500ml graduated cylinder and add H<sub>2</sub>O to 500ml.*

**Next, let's look at how to make other solutions from our stock solutions:**

You have been taught the formula  $C_1V_1 = C_2V_2$ .

Here's the formula that I have used for years in the lab, and it is really just the same equation in a logical order, with the order to punch it in the calculator:

"What you have (stock conc)  $\div$  what you want (final conc); invert that under 1 with the  $\frac{1}{x}$  key, then multiply by the volume you want to make."

That also looks like this: 
$$\frac{\text{final volume}}{(\text{stock conc}/\text{final conc})} \leftarrow \text{dilution factor}$$

That's the same as the  $C_1V_1 = C_2V_2$  formula, solving for  $V_2$ . The (stock conc. / final conc.) is also known as your "dilution factor."

So, let's say you already have stock solutions of 5M NaCl; 1M Tris, pH 8; and 1M Imidizole, and you need to make 500ml of 100mM NaCl, 10mM Tris, pH 8, and 20mM Imidizole. Here's how you do the calculations:

Step:	NaCl	Tris, pH 8	Imidizole
Have $\rightarrow$ Need	5M $\rightarrow$ 100mM	1M $\rightarrow$ 10mM	1M $\rightarrow$ 20mM
(get same units)	5000mM $\rightarrow$ 100mM	1000mM $\rightarrow$ 10mM	1000mM $\rightarrow$ 20mM
Have/need = Dil Fctr	1:50 dilution	1:100 dilution	1:50 dilution
scale up:	10:500 dilution	5:500 dilution	10:500 dilution
add units:	10ml stock:500ml total	5ml stock:500ml total	10ml stock:500ml total

Plugging in the calculator for NaCl would be:

5000  $\div$  100  $\frac{1}{x}$  500  $=$ , and the result will be 10, or 10ml. Try this method for the other 2 solutions above.

The description of how to make the solution is this:

*To a graduated cylinder, measure by pipette 10ml 5M NaCl, 5ml 1M Tris, pH 8.2, 10ml 1M Imidizole, and water to a total volume of 500ml.*

Since all the stock solutions are already liquid, there is none of that tedious "Stir to dissolve" or transferring to a graduated cylinder later; the solution can be made directly in the grad cylinder in the first place, and brought to final volume in one easy step. It will mix as you pour it into your bottle for storage (which you will clearly label...but that's another lesson!)

You can do the same for %-solutions: suppose you need to make 100ml of a solution with a final concentration of 1% SDS, and you already have a bottle of 20% stock on your shelf.

20%  $\rightarrow$  1% SDS = 1:20 dilution, and that's the same as 5:100, so you would use 5ml of the 20% stock in the total volume of 100ml.

Plugging in the calculator would be:

20  $\div$  1  $\frac{1}{x}$  100  $=$ , and the result will be 5, or 5ml in a total of 100ml.

The next type of solution we use in the lab is an **X-solution**. This is a solution that someone has worked out in the past which may have several fairly dilute components, and we have found it is generally easier to measure out 10-times the amount of each and prepare the stock solution in a

more concentrated strength. For use, the 10X solution is diluted out to 1X or 0.5X, and is labeled as such.

For example, a common X-solution is 10X PBS (PBS stands for Phosphate-Buffered Saline). The 1X concentrations are listed as:

137mM NaCl,  
2.7mM KCl,  
10mM Na<sub>2</sub>HPO<sub>4</sub>,  
2mM KH<sub>2</sub>PO<sub>4</sub>.

The recipe for this would be 8g NaCl, 0.2g KCl, 1.44g Na<sub>2</sub>HPO<sub>4</sub>, and 0.24g KH<sub>2</sub>PO<sub>4</sub> per 1000ml total volume, pH 7.4. (*You should practice those calculations yourself, using MWs of 58.44 for NaCl, 74.55 for KCl, 141.96 for Na<sub>2</sub>HPO<sub>4</sub> and 136.09 for KH<sub>2</sub>PO<sub>4</sub>.*) PBS is generally used in large volumes (500ml-1000ml at a time), so it would be a waste of time to make 1 liter of 1X PBS over and over again. However, making a concentrated (10X) solution is more time efficient; the larger masses are easier to weigh out; the pH titration is done only once per 10 liters total volume if the solution is made at the 10X concentration. Additionally, it is a simple process to dilute the 10X solution to 1X as it is needed.

So, to prepare 1L of 10X PBS (notice how the weights are all 10x as listed above):

*Dissolve 80g NaCl, 2g KCl, 14.4g Na<sub>2</sub>HPO<sub>4</sub>, and 2.4g KH<sub>2</sub>PO<sub>4</sub> in 950ml H<sub>2</sub>O, and titrate to pH 7.4 with acid. Pour the solution into a 1000ml graduated cylinder, and add H<sub>2</sub>O to a total volume of 1000ml.*

{This particular solution needs to be sterilized by autoclaving or filter sterilization before use and storage, so you would either pour it into a sterile bottle through a 0.22um sterile filter, or put it into glass bottles and autoclave it for 20 minutes at 15psi on the liquid cycle.}

There is one other type of solution to make, and is mostly used for things like antibiotics: **it is a simple mg/ml or µg/ml solution (the same as a w/v solution)**. For instance, Ampicillin is generally made at a stock concentration of 50mg/ml. Suppose you need to make some of this stock for use over the next few months. I'd make at least 50ml, so the calculation is like this:  
 $50\text{mg/ml} = 500\text{mg}/10\text{ml} = 2500\text{mg}/50\text{ml}$ , or 2.5g/50ml.

You would weigh out 2500mg of Ampicillin, put it into a 50ml graduated cylinder, and add water to 50ml. Capping and sealing the cylinder with Parafilm (a handy lab supply) allows you to rock or invert the cylinder in your hand until the powder is dissolved. Even though the antibiotic does kill non-resistant bacteria, it needs to be sterile-filtered (autoclaving it would inactivate its activity) to be used in sterile plates and media.

Once you have your sterile stock of 50mg/ml, you find that you need to make some LB media with Ampicillin at 50µg/ml; diluting mg to µg is a 1:1000 dilution. So if you want to make 25ml of LB with Ampicillin at 50µg/ml, you would add 1µl Amp per ml of media (that's a 1:1000 dilution), or 25µl Amp/25ml LB. The volume of Ampicillin being added (25µl) is inconsequential to the total volume (25ml), so don't worry about off-setting that in the preparation.