Experiment 1

Calculations in biochemistry

Preparing and handling solutions is an essential part of experimental biochemistry. One of the key tools any new science graduate can have is to be competent in preparing reagents, buffers, and accuracy in pipetting. The three most common concentration expressions biochemists use are molarity, normality, percent (vol/vol, wt/vol, mg% and wt/wt) and ”X” concentration.

SOLUTIONS: Several methods have been developed for the preparation of solutions, the following methods are used in biochemistry:

1. Percent solution. (%).
2. Molar solution (Molarity)
3. Normality
4. ”X” concentration.

Stock solutions (or concentrated solutions) are often defined as percent, Molar, Normal or the informal “X” concentration. If a solution is made of one component, then the molarity is usually shown. When a number of chemicals are used to make a concentrated solution, the concentration factor X is used.

PREPERATION OF SOLUTIONS:

1. Concentrations in percent:

A. Percent by weight (w/w)

(X g of solute/100 g of solvent)*100 = X%

Ex. a 10% (wt/wt) solution would be 10 g of solute and 90 g of solvent.

Example
A pharmacist asks a pharmacy technician to remove 30 gm of hydrocortisone from a tube of 1% hydrocortisone cream. How many grams of hydrocortisone are in 30 gm of the 1% product?

Set up a proportion → 1 gm : 100 gm :: X : 30 gm

Solve: (100)(X) = (30)(1) → 100X = 30 → X = 30 ÷ 100 = 0.3 gm

Therefore, 30 gm of a 1% product contains 0.3 gm of hydrocortisone.

Fig. 25

B. Percent by volume (v/v)

(X ml/100 ml of total solution)*100 = X%

Ex. A 10% (v/v) solution would be made by adding 10 ml of concentrated solution to 90 ml of dilutent.

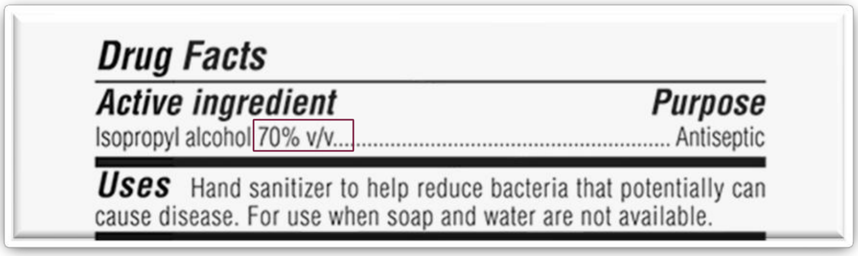
Example
In the preparation of 200 mL of a topical product, the pharmacist added 6 mL of liquefied phenol. What is the percentage (v/v) of liquefied phenol in the topical product?

In other words, if there are 6 mL of phenol in 200 ml, how much phenol is in 100 mL of this solution? This will give you the v/v percentage for the solution.

Solve by setting up a fractional equation and cross multiply.

$\frac{6\ mL}{200\ mL} = \frac{X}{100\ ml}$ → (200)(X) = (100)(6) → X = 600 ÷ 200 = 3 mL in 100 mL or 3%

Fig. 24



C. Percent by weight per volume (w/v)

(Xg of solute/100 ml total volume)*100 = X%

Ex. A 30% NaCl solution would be prepared by adding 30 g of salt to a vessel containing 50 ml of water then QS (*fill to **Quantity Sufficient***) to measure 100ml.

Do not add 30 g to 100 ml of water, the resulting solution would be more dilute than planned.

Example
How many grams of sodium chloride are in 500 mL of 0.9% NaCl solution?

$\frac{0.9\ gm}{100\ mL}$ x 500 mL = (0.9 x 500) ÷ 100 mL = 4.5 gm of sodium chloride

Using a fractional equation:

$\frac{0.9\ gm}{100\ mL} = \frac{X}{500\ mL}$ 500 mL x 0.9 gm = 100 mL x X → 450 = 100X → X = 4.5 gm sodium chloride

Example
How many grams of dextrose are required to prepare 3000 mL of a 10% solution? Remember 10% means 10 gm in 100 mL of solution.

3000 ~~mL~~ x $\frac{10\ gm}{100\ \text{mL}}$ = (3000)(10) ÷ 100 = 30,000 ÷ 100 = 300 gm

Therefore, 300 gm of dextrose is needed to prepare 3000 mL of a 10% solution.

Fig. 23

D. Percent by mg per volume (mg %)

mg of solution/100 ml of total volume

Milligram % is often used in clinical laboratories. For example, a clinical blood sugar value of 225 means 225 mg of glucose per 100 ml of blood serum.

- Somehow similar to w/v, however here we use mg/100ml, rather than g/100ml.

2. <u>Concentration in molarity</u>

Molarity is the most common concentration unit in biochemistry. Make certain that it the abbreviation is a capital M. *Brackets [ ] indicate molar concentration, usually in M.*

$$\text{Molarity (M)} = \frac{\textit{Number of Moles}}{1\ \textit{liter of solution}} = \frac{\frac{\textit{gram of solute}}{\textit{molecular weight}}}{1\ \textit{liter of solution}}$$

Example: How many moles of NaCl are present in 150 mL of a 1.5 M solution?

number of moles = M x V

$$\text{where V} = \frac{150\ ml}{1000\ ml/L} = 0.15\ \text{L, and M} = 1.5\ \text{moles/L}$$

number of moles = 1.5 moles/L x 0.150 L

= 0.225 mole NaCl;

MW NaCl= 58.5 g/mole, which is also equal to 0.225 x 58.5 = 13.1125 g NaCl.

Dilute solutions are often expressed in terms of a smaller unit therefore,

1mM $=10^{-3}$ M = 1mmole/liter = 1 µmole/ml

1µM = 10^{-6} M = 1µmole/liter = 1nmole/ml

1 nM $=10^{-9}$ M = 1nmole/liter = 1pmole/ml

If you divide both units by 1000
This will convert the units from liter to ml
and from mmol to µmole

3. Concentration in normality

Normality refers to the concentration of a solution expressed in terms of the number of equivalents of solute in 1 L of solution. This is same as the number of milliequivalent weights (milligram-equivalent weights, milli equivalents “meq”) in one mL of solution. Normality is indicated by the symbol **N** and is used, in calculations involving acid-base (neutralization) and oxidation-reduction (redox) reactions.

$$\text{Normality (N)} = \frac{Number\ of\ equivalents}{Volume\ (1\ liter)}$$

No. of equivalent = (weight ÷ EW)

EW= molecular weight/ n

The EW is the mass of one mole of ion (either H+ or OH-)

Where n is the number of replaceable H+ or OH- per molecule for acids and bases

$$N = \left(n * \frac{weight}{molecular\ weight}\right) / \text{ volume in litre}$$

N= [(n * (weight/ molecular weight)]/ volume litre

N= [n * no of moles]/volume in litre

N= n * M

* While Molarity refers to the concentration of a compound or ion in a solution, normality refers to the molar concentration only of the acid component or only of the base component of the solution. Thus, **normality offers a more in-depth understanding of the solution's concentration in acid-base reactions**.

Example:

Calculate the no of moles and molarity of 0.5 N solution of H2SO4 made using 500 ml?

N= n* M

0.5= 2* M -------> M= 0.5/2= 0.25 M

M=No of moles/ volume in litre

No of moles= M* volume in litre

= 0.25* (500/1000)

= 0.125 mole.

Dilutions

It is often necessary to change the concentrations of one solution to that of a more dilute solution. In the process of dilution, the total amount of solute remains unchanged, only its concentration is decreased. This can be done by utilizing the fundamental relationship just as follows:

$$C_1 \times V_1 = C_2 \times V_2$$

where:

C1 = concentration of initial solution.

V1 = volume of initial solution.

C2 = concentration of desired (final, diluted) solution.

V2 = volume of desired (final, diluted) solution.

- This calculation is used for diluting concentrated solutions, usually those that are in percent, molar, or normality units, also X factor can be used.
- It is NOT intended to be used when dealing with serial type of dilutions.
- However, it is very easy to use, but can be a large source of error for if the calculations are not performed correctly. The biggest problem in using this method is when you don't keep the units the same. When using millilitres on one side of the equation then you need to use millilitres on the other. The same goes for concentration units.

When additional diluent is added to an aqueous solution, the concentration of that solution decreases. This is because **the number of moles of the solute does not change**, while the volume of the solution increases. **the number of moles are the same before and after dilution**.

No of moles= no of moles

M1 x V1 = M2 x V2

The two beakers contain the same number of moles of solute.

Example: Prepare 50.0 mL of a 2.0 N solution from a 5.0 N stock solution.

$C1 \times V1 = C2 \times V2$

$5.0 \times V_1 = 2.0 \times 50$

$V_1 = 20.0$ mL

To 20.0 mL of stock solution add 30.0 mL of diluent.

Example: Dilute 7.0 mL of a 5.0% (w/v) solution to a 3.0% (w/v) solution.

$C1 \times V1 = C2 \times V2$

$5.0\% \times 7.0 = 3.0\% \times V_2$

$V_2 = 11.7$ mL

To 7.00 mL of initial solution add 4.7 mL of diluent.

4. The "X" Factor

When a solution contains a mixture (more than one chemical) in a solution, the X factor comes into play. The buffers used in biochemistry laboratory usually have three or four components. Instead of listing the molar concentration of each chemical in the solution, a stock concentrated solution might be labelled as 10X Buffer. This tells the researcher that the stock solution is ten times more concentrated than it needs to be for use. Thus, a dilution is in order.

Components	**1X (working concentration)**	**10X (stock concentration)**
A	**1 M**	**10 M**
B	**0.5 M**	**5 M**
C	**0.3 M**	**3 M**
D	**2% w/v**	**20% w/v**
E	**0.1% v/v**	**1% v/v**

When making a concentrated stock solution (e.g 10X buffer) you need to know the "working" concentration which is the concentration of each of the chemicals when the solution is at the correct concentration to use. This is called 1X. All concentrated stock solutions that use the X factor, uses the working concentration as 1 X. However sometimes it can be 2X or 5X.

Volume of stock solution (A) needed = $\frac{\textbf{Volume of 1X needed}}{\textbf{Concentration factor}}$

Let's say you need 50 ml of 1X running buffer to perform an experiment and the stock concentration is 50X.

$= \frac{50\ ml}{50\ X}$ = 1 ml is required from A + 49 ml diluent.

طيب هلا أنا لو بدي أجي حدا حكى لي يا 1 dilution to 10 طيب 1 dilution to 10 بدي أحكي أنا بدي أخذ جزء وأضيفه مع 9 أجزاء. طيب هلا بالعادة مثلا ممكن ما يكون أنا بحكي لي أحضر مثلا 10 مل، ممكن 20 مل، ممكن 30 مل، ممكن 40 مل، فبدي أحسب كم هو الجزء من 10 صح؟ إذا أنا مثلا عندي 30 مل، إذا عندي 40 مل، كم الجزء من 10؟ وكم 9 أجزاء من 10 هدول كم بمثلوا عندي volume؟ أوكي؟ معناها أنا لما مثلا يجي حدا يحكيلي 1 dilution of 1 to X مثلا 1 to 20, 1 to 10, 1 to 5، معناها أنا ال concentrated solution should be diluted to 1X of its current concentration. فبجي بحسب، بحكي ال volume of each part تساوي total volume على total parts اللي هي X مثلا أنا حدا بيحكيلي 1 to 10، معناها أنا عندي 10 total parts، وحكى لي مثلا حضري 100 مل، فبحكي 100 على 10، معناها كل part هو عبارة عن 10 مل. أوكي؟ هلا بدي أحكي أنا في عندي بدي أخد من concentrate المركز اللي هو 10 مل، بدي أضيفه، بدي أضيف عليه يعني add the concentrated solution to X minus 1 volume of the diluent. فأنا كم عندي X minus 1؟ 10 ناقص 1 9. أوكي؟ معناها عندي 90 مل وبضيفهم على 10 مل من ال concentrated solution. أوكي؟ وبالعادة دائما we add the small amount to the large amount يعني بكون حاطة 90 مل بالأول عندي بال beaker أو بال volumetric flask بعديها ببلش أضيف عليها ال 10 ML من ال concentrated solution.

Or you can use

C1 * V1 = C2 * V2

50X * V1= 1X* 50 ml → V1 = 1 ml is required from A + 49 ml diluent.

❖ Dilutions

There are two main methods for interpreting dilutions 1 to 10

a) means that there is one part of concentrate in 10 parts of final solution (e.g. 1 ml of concentrate and 9 ml solvent with a final volume of 10 ml)

b) means that there is one part of concentrate to 10 parts of solvent (or 1 in 11) (e.g. 1 ml of concentrate and 10 ml vehicle with a final volume of 11 ml).

The former convention (one part / total parts) is frequently used because the dilution factory is easy to work with. In this case it is best to read "dilute 1:10" meaning "add one part of the sample plus nine parts of diluent". Now it is easier to determine the calculations.

If you wish determine the volumes of each part. A dilution of 1:X means your concentrated solution should be diluted to 1Xth of its current concentration.

$$\text{Volume of each part.} = \frac{total\ volume}{total\ parts\ "X"}$$

Add the concentrated solution to (X-1) volumes of diluent. ***Always add a small amount to a larger amount.***

هلأ في عندي طريقتين، إذا أنا بدي أترجم جملة one to ten أو one نقطتين رئيسية ten ten أوكي؟ هلأ ممكن نفهمها على جهتين الجهة الأولى إنه أنا في عندي one part موجود in ten part as a total يعني أنا مثلاً لو بدي أخذ واحد mlهي عبارة عن واحد ml من ال concentrate المحلول المركّز زائد تسعة ml من ال solvent فبالتالي الfinal volume هو عندي عشرةmlممكن كمان تنفهم إنه أنا عندي مثلاً one part من ال concentrate plus عشرة parts من ال vehicle أو ال solvent اللي أنا بدي أستخدمه فبالتالي final volume عندي بصير 11 هلأ بالعادة ال former convection اللي هو الخيار الأول اللي هو A بنستخدمه بشكل more frequent لأنه أسهل في الاستخدام لما بجي بحكي one to ten أنا دائماً ببالي إنه final volume عشرة مش مضطر أصير أجمع الواحد مع العشرة عشان أحكي إنه final volume عندي كم إنه بصير 11 فبالعادة ال best case أو it's best to read it as one to ten معناها إني أنا أضيف one part of the sample plus nine parts of the diluent

أولًا: القاعدة العامة لأي 1 : X Dilution

إذا قيل لك:

$$1 : X$$

فهذا يعني:

- عدد الأجزاء الكلي = X
- جزء واحد من الـ concentrate
- و (X − 1) أجزاء من الـ diluent
- الحجم النهائي = X أجزاء

الطريقة الحسابية العامة

إذا طُلب منك تحضير **حجم نهائي معيّن (V total)**:

الخطوة 1️⃣

احسبي حجم الجزء الواحد:

$$\text{Volume of one part} = \frac{V_{total}}{X}$$

الخطوة 2️⃣

- حجم الـ concentrate = جزء واحد = V_{total}/X
- حجم الـ diluent = $V_{total} - (V_{total}/X)$

أو مباشرة:

$$\text{Diluent volume} = \frac{X-1}{X} \times V_{total}$$

مثال 1️⃣

1 : 10 dilution 100 وتحضير mL

- X = 10
- Vtotal = 100 mL

حجم الجزء الواحد:

100 ÷ 10 = 10 mL

إذن:

- Concentrate = 10 mL
- Diluent = 90 mL

✓ الحجم النهائي = 100 mL

مثال 2️⃣

1 : 10 وتحضير 30 mL

30 ÷ 10 = 3 mL

- Concentrate = 3 mL
- Diluent = 27 mL

مثال 3️⃣

1 : 10 وتحضير 40 mL

40 ÷ 10 = 4 mL

- Concentrate = 4 mL
- Diluent = 36 mL

نقطة مهمة جدًا 🎯

عندما يُقال:

1 to X dilution

هذا يعني:

> تركيز المحلول النهائي أصبح X/1 من تركيزه الأصلي.

مثلاً:

- 1:10 → التركيز صار 1/10 من الأصلي
- 1:20 → التركيز صار 1/20 من الأصلي
- 1:5 → التركيز صار 1/5 من الأصلي

نقطة عملية في المختبر 🧪

كما ذكرتِ تمامًا 👌

بالعادة:

> نضع الحجم الأكبر أولًا (diluent)، ثم نضيف الحجم الأصغر (concentrate).

مثلاً في 1:10 لتحضير 100 mL:

- نضع 90 mL diluent
- ثم نضيف 10 mL concentrate

هذا أدق وأسهل في القياس.

ملخص سريع تحفظيه للامتحان 📌

إذا أعطاكِ 1 : X وتريدين حجم نهائي V:

Example: a dilution of 1:5, means that the concentrated solution should be diluted to 5th of its current concentration

I have 100 ml solution of concentration 10%,

Volume of each part= 100/5= 20 ml for each part

Add 20 ml from concentrated stock to X-1 parts

i.e. 5-1 = 4 parts

4 parts* volume of each part

= 4* 20 ml = 80 ml diluent (final solution concentration is 2%)

❖ **Serial dilution:**

Serial dilution is used when you need a volume or amount that is too small to measure (example, prepare 0.00007 mg/ml, there are no balances to measure this small weight amount), options in this case is:

إما إني أنا راح أضطر آخذ وزنة اللي هي ال minimum اللي بستطيع يوزنها الميزان وأضيف عليها كمية volume كبيرة جداً، أضطر أحضر 100 لتر أو 200 لتر عشان أحصل على التركيز هذا، أو إني إيش بعمل؟ بروح بحضر تركيز عالي ولكن بحجم قليل، أوكي، بعدها بعمل multiple dilution steps، بسميها serial dilution، عشان أوصل للتركيز اللي أنا بدي إياه. هلا ال serial dilution بسميه stepwise serial dilution، small amount of start material، بنضل نعمل application dilution، يعني بصير في عندي ال dilution factor يكبر، كل مرة بخفف بصير في عندي ال dilution factor يكبر، لحد ما أوصل للتركيز اللي أنا بدي إياه

1- Prepare larger volumes than what you need.

2- Make a small volume of a higher concentration and then perform multiple dilution steps (serial dilution) to reach the required concentration. A serial dilution is a stepwise series of dilutions which starts with a small amount of starting material and amplifies the dilution factor serially by using diluted material as a source for subsequent dilutions.

Serial dilution advantages include:

1- Saving reagents/spaces

2- Used in experiments which require standard curve.

كيف إنه نحن بنحضر شيء اسمه standard curve، إني أنا مثلاً باجي بدي أحضر خمس تراكيز من مادة معينة عشان أرسم منها علاقة خطية وأستطيع إني أحصل على تراكيز مجهولة أنا مش عارفيتها.

✓ Serial two-fold and ten-fold dilutions are commonly used to prepare diluted analytes. Serial dilutions are also commonly used to avoid having to pipette very small volumes

1 mL من ال stock

أختي:

وضفتي 9 mL ماء

يعني الحجم النهائي = 10 mL

إذن:

DF = \frac{10}{1} = 10

يعني خففنا المحلول 10 مرات

فنسميها:

1:10 dilution

أو ten-fold dilution

بعمل هيك 👍

1.بالبداية بحضر تركيز متوسط بحجم مريح (مثلاً بال mL)

2.بعدين ببلش أخفف خطوة خطوة.

3.بكل خطوة بصير أسحب أحجام طبيعية وسهلة (1 mL – 0.5 mL مثلاً).

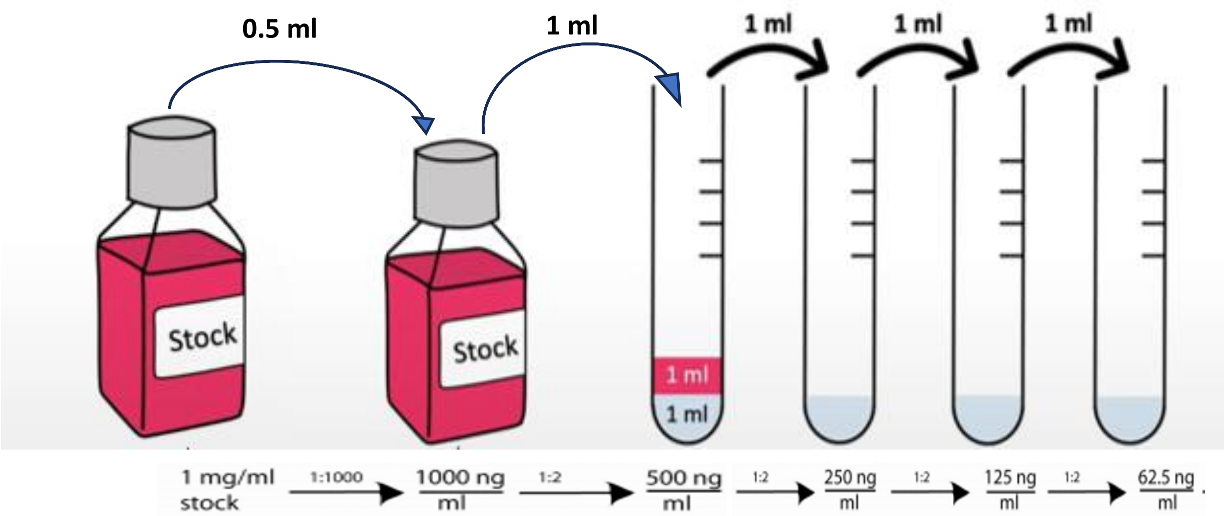
4.لحد ما أوصل للتركيز الصغير المطلوب

Example:

I want to prepare various concentration of solution Y in the range of 1000 ng/ml to about 60 ng/ml, and the stock concentration is 1 mg/ml

Answer: we cannot weigh any amount in nanogram, therefore a serial dilution is required.

1- The first step: dilute the stock to get the highest primary concentration required (1000 ng/ml). This will be done by performing an initial 1: 1000 dilution.



To prepare 500 ml of 1000 ng/ml (you can prepare any volume, e.g. 1000 ml, 250 ml, as long as your concentration calculation is correct):

C1 * V1 = C2 * V2

1,000,000 ng/ml * V1= 1000 ng/ml* 500 ml → V1 = 0.5 ml is required from stock + 499.5 ml diluent.

OR

$$\frac{C1}{C2} = dilution factor$$

$$\frac{1,000,000\ ng/ml}{1,000\ ng/ml} = 1000\ dilution factor$$

Then divide the volume required by the dilution factor:

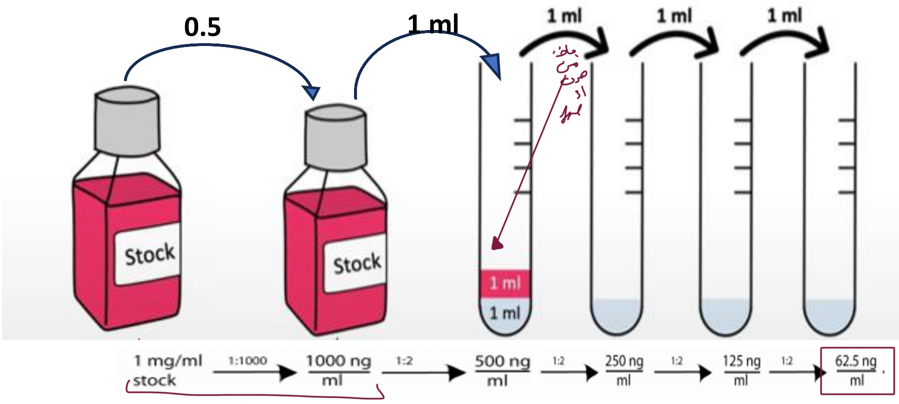
= 500 ml/1000= 0.5 ml from stock + 499.5 ml diluent.

مرة بنأخذ نصف حجم المحلول السابق ونضيف نفس الحجم من الديليوت → تركيزه يصير نص السابق
دف: نغطي كل الرينج المطلوب بدقة وسهولة بدون ما نضطر نزن كميات صغيرة جدًا على الميز

2- To obtain the range required, **two-fold dilutions** can be performed.

For the first 1:2 dilution we would take 1 ml of the 1,000 ng/ml solution and add it to 1 ml of diluent in the next tube. This will result in a solution with a concentration of 500 ng/ml.

This solution is then used in a subsequent or serial dilution by taking 1 ml of the previous 500 ng/ml solution and adding it to the next tube containing ml of diluent. This dilution will now result in a solution with a concentration of 250 ng/ml. This process is repeated until a final solution at a concentration near 60 ng/ml is made. In the example below, the highest dilution has a concentration of 62.5 ng/ml.



The total dilution factor for any of the resulting solutions can be calculated by multiplying each dilution factor leading up to a particular dilution. For example if we wanted to find the total dilution for the 125 ng/ml solution:

كل خطوة تخفيف لها DF:
الخطوة الأولى (من الستوك 1 mg/mL إلى 1000 ng/mL) →
DF = 1000
كل تخفيف ثانوي 1:2 → DF = 2
لحساب الـ DF النهائي لأي أنبوب، نضرب كل DF سابقة

$$DF_{total} = DF1 \times DF2 \times DF3 \times DF4$$

$$DF_{total} = 1000 \times 2 \times 2 \times 2$$

$$DF_{total} = 8000$$

You can use the DF*total* to calculate the concentration from the stock. The stock is 1,000,000 ng/ml/8,000=125 ng/ml.

Note: you can use various volumes, add 0.5 ml from the dilution to 0.5ml, or 1 ml from the dilution you want to 1 ml, or 2 ml from the dilution you want to 2 ml, this depends on the volume you want for your experiment, and the diluent quantity available in your lab.

YouTube Link:

Serial dilution

https://www.youtube.com/watch?v=yYWFX4IXc5Y

Drug reconstitution

https://www.youtube.com/watch?v=8ECYuiHFObU

Dosage Calculations Made Easy

https://www.youtube.com/watch?v=TK3ZAaMuhYkhttps://www.youtube.com/watch?v=TK3ZAaMuhYk

Experiment 2

Experimental applications on solution preparation and dilution.

In this laboratory you will be preparing and diluting solution as per given in the lab. In all steps distilled water will be used as a diluent

Using the following materials, prepare the solution below

Materials

- NaCl
- KCl
- Red buffer
- 10% bromophenol blue stock (3mls)
- H_2SO_4
- Volumetric flasks (50 mL)
- Digital balance
- Graduated pipettes
- Pipette filler.
- Distilled water
- Test tubes
- Erlenmeyer flask
- Beaker

1- Prepare 4 mls of 0.078% of bromophenol blue, given that the stock concentration is 10% bromophenol blue. Write down the procedure you followed in your report sheet.

2- Prepare 50 ml of 20% w/v NaCl?

3- Prepare 50 ml of 136.8 mM NaCl using the stock solution (20 % w/v) you prepared in the last step, knowing that the molecular weight of NaCl is 58.44 g/mole.

4- You have a 10X red buffer, prepare 10 ml of 1X buffer.

5- Prepare 50 ml of 150 mM KCl knowing that the molecular weight of KCl is 74.5513

6- Prepare 50 ml of 0.2 N H_2SO_4, from a stock of 2 M H_2SO_4 found in the fume hood?