Experimental Biochemistry
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Lecture – 03
Introduction to Biochemistry Laboratory and Safety Measures

Hello everybody, I am Mister Aditya Jyoti Basak, Senior research fellow working under the supervision of Professor Dikbindu Shamanto and Professor Shomad (Refer Time: 00:27) at the School of Bioscience, Indian Institute of Technology Kharagpur. I am one of the designated teaching assistants who will be guiding you through this online NPTEL experimental biochemistry course. See you often. So, before we move on to new experiments and learn new techniques what I would like to do is, I would like to show you some of the common instruments that are available in any well setup molecular biology and biochemistry laboratory.

So, first of all I would like to show you this instrument here.

(Refer Slide Time: 00:58)

So, this instrument is a laminar air flow cabinet and you will be looking at this instrument later on in further detail in the subsequent lectures, but in general what this instrument is used for, is basically once you open this and it is switched on. There is an

airflow filter and it maintains an airflow positive pressure, which prevents outside air from entering this enclosed area and this enclosed area is sterile.

So, there are UV lamps which sterilize the inner region when not in use and also we keep spirit lamps and burners, where we light them and we heat sterilize and alcohol sterilize this entire region. So, this cabinet is mainly useful when you are working with bacterial cultures and you want to grow your bacteria. But you want to make sure that no other bacteria is growing apart from the bacteria of your interest. So, to maintain sterile environment, we use this laminar airflow cabinet.

(Refer Slide Time: 01:58)

So, the machine that I have in front of me now is a temperature control shaker incubator and the purpose of this is that it allows us to grow bacteria in a controlled temperature environment. As you can see inside there are different flasks containing volumes of bacterial growth media, in which bacteria have been inoculated and as time progresses, they will grow in a temperature controlled environment.

So, for now it has been set at 37 degree centigrade and the shaking is essential, because it prevents the bacteria from settling down which is detrimental to bacterial growth and even more importantly, it ensures proper aeration of the culture so, that the bacteria can survive. So, some of the salient features are for now the bacteria that we are growing today is at 37 degree.

But in many instances scientists need to grow bacteria at a lower temperature. In case the protein that we need to express is toxic or in case the protein that is being expressed is not properly soluble, for such instances bacteria needs to be grown at maybe 16 degree or 25 degree and those temperatures can also be set and maintained by this shaker incubator. And also the RPM for now it is revolving at 200 revolutions per minute, but that can also be lowered or increased as per requirement. So, you will be learning in detail about these experiments in the subsequent lectures.

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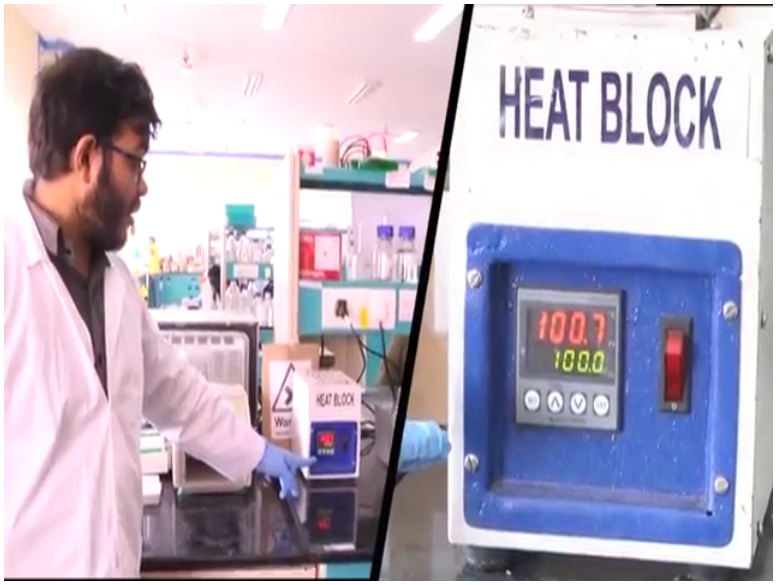

So, for now what I did was, just gave you a flavor of the instruments that are necessary for carrying out work in molecular biology and biochemistry. So, for certain experiments, it is necessary to maintain the temperature of the reaction at 100 degree centigrade, for which we have a dry heat block here, you take your sample which you want to maintain at 100 degree and you just place it here like this and then it will be heated to 100 degree centigrade and will be maintained at that for; however, long you.

(Refer Slide Time: 04:05)

You have already been introduced to the dry heat block, but apart from that we also have water baths in our laboratory where; so, this is the water bath. And so, this portion of the tank has water which is maintained at for now it is maintained at 42 degree centigrade, that is because later on today what I will be doing is, I will be doing an experiment in which I require the temperature to be at 42 degree. I will take my sample in a centrifuge tube and I will just keep it inside this water bath. It will float inside the water bath and the reaction will be incubated at 42 degrees. So, not just 42 degree for some purposes, it might be required at 56 degrees, for some purposes it might be required at 35 degrees. So, you can adjust the temperature and use the water bath for that sort of purpose.

(Refer Slide Time: 04:59)

So, what we have here is a gel rocker. So, basically as you can see this is an instrument which has a rocking platform on which we keep our different boxes. So, inside our boxes we already have a different sds page gels and you see a blue liquid inside the box right. So, the gel is kept and on the top of that we have added the stain or the d stain, and this uniform rocking motion ensures that the entire gel is being properly exposed to the stained solution. Apart from that sometimes when we need to coat a surface with an antibody what we can do is, we can take that in a reaction tube and leave it here and the in the rocking motion caused by this instrument ensures proper mixing.

So, I am I understand that you might not be familiar with many of the experimental terms that I used, but that is not the point for the at this moment. What I want you to realize is, we have instruments which ensure proper mixing of different liquids on to solid surfaces or between each of them via this gel rocker.

(Refer Slide Time: 06:08)

What I have in front of me now is a centrifuge, it is a temperature control centrifuge and its purpose is to centrifuge different samples. And this is a temperature control centrifuge, which means we can maintain it at 25 degree centigrade or lower 24 degrees or 16 degrees or even if required slightly higher.

So, we can centrifuge solutions using such centrifuges and you will be seeing in the subsequent lectures that centrifugation is a very essential process required for many different experimental purposes in the laboratory. And so, this is just one sort of centrifuge as we will be showing you there are different types of centrifuges and the principle of centrifugation remains the same. The only difference is some of them like this one might be refrigerated, some might not be refrigerated, some of them can handle larger sample volumes, some of them can handle very small sample volumes and so, on.

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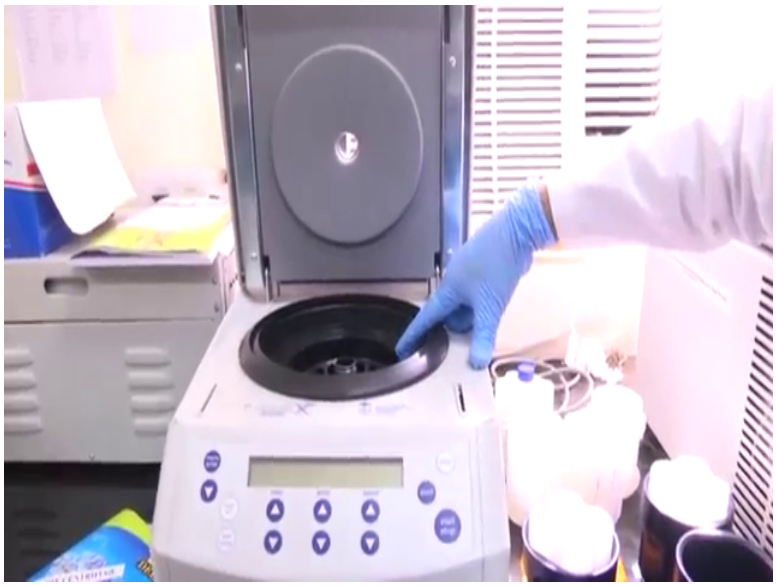

So, the earlier centrifuge that I showed you had a swing out bucket, I could not show it to you because the machine was running, but here what you can see in this centrifuge is it has a fixed angle rotor.

(Refer Slide Time: 07:25)

So, as you can see these small holes, inside these holes we can play small eppendorf tubes and right now the machine is switched off, but what will happen is once I put the centrifuge tubes here the machine rotates. And as you can see the centrifuge tube which has been placed here or which will be placed here will always remain at a constant angle.

So, that is why this is called a fixed angle rotor and so, this centrifuge works in a similar fashion to the earlier centrifuge, and I will be showing you some other centrifuges subsequently.

(Refer Slide Time: 08:00)

This centrifuge here has a different type of rotor. So, till now what you have seen is, centrifuge rotors which can accommodate centrifuge tubes 1.5 ml centrifuge tubes, there are rotors which can accommodate 15 ml falcon tubes or 50 ml conical falcon tubes. But this rotor that I will be showing you here is a bit different because this can actually accommodate 96 well plates.

(Refer Slide Time: 08:25)

For many type of experimental purposes you might need to centrifuge samples kept inside 96 well plates and for that purpose, this sort of centrifuge rotor is necessary.

(Refer Slide Time: 08:45)

For certain experiments, it is necessary to carry out the reactions under ice cold conditions for that we need ice makers or ice flake machines. So, this small instrument here does exactly that, it is connected to a water supply source and what it does is, it creates flake ice for us; let me just show you. So, as you can see I just took out some ice. So, flaked ice, which we take inside these ice buckets and so, this is a very

underappreciated, but very essential component of the laboratory. Because if you do not have ice cold conditions many reactions will not work and making ice cubes on in normal refrigerators is laborious and time consuming and does not really suit the purpose.

So, often it is quite essential to have ice flake machines, which will take water supply directly from the tap and they will convert it into flake dyes which you can then just take out in use.

(Refer Slide Time: 09:46)

Another very important and basic requirement for molecular biology or biochemical experimental work is the usage of proper quality of water. You cannot just use tap water and carry out different reactions or carry out different experiments, because you do not know what ions or minerals or what the pH of the water in the tap is.

So, for that purpose you will require de ionized water and what I have in front of me are a set of different instruments this one, this one that one all of which are working in tandem, to provide us with de ionized water purified de ionized water. So, I will not talk too much about the internal components, but basically what it does is, the tap water source that is coming to the laboratory initially the as you can see here iron removal cylinder is here.

So, what it does is, it removes the iron and other heavy impurities heavy metal impurities. Apart from that, inside this component of the water filtration system there are filters having pore sizes of maybe 5 micron or 2 micron or both. And what they do is, they remove the large sized particles, they remove the sedimented particles, they remove impurities from the water. Apart from that inside this machine they carry out the deionization. So, they remove all the ions using different expensive membrane filters.

(Refer Slide Time: 11:28)

And finally, the purified de ionized water that is present is stored in this tank from which we can take as per our requirement.

So, this is the general principle we have a particular company's water purification unit, but there are different companies which build similar instruments and they all work on the same basic principle. And always keep in mind that the water which we are using for setting up your experimental reactions needs to be de ionized, it should not just be taken from the tap and used directly.

So, now, I am going to demonstrate how to actually use a pipette in the lab.

(Refer Slide Time: 12:10)

So, I am going to take 100 to 1000 micro liter pipette and let us say I want to collect 1 ml of a particular solution and then dispense it into my desired container. It is first of all lets set it at 1000 suppose it was set lesser, we just go up and set it at 10000 micro liters. Whenever you are working with lipids always ensure that you are holding it in this position do not hold it like this or do not hold it like this while the pipette is affixed, because that could lead to liquids rolling in your instrument always hold it like this.

Set it at the proper volume and then what you need to do is you take out your tip box notice what I am doing here, you slide down the lower portion of your pipette it will fit very nicely into the tip, give a slight twisting motion while you are pressing down. And the tip will automatically get attached to the pipette. You lift it up and you have your tip attached to your pipette line.

So, another thing that you need to know is, this piston can go up and down depending on the pressure that I exert with my thumb. So, when you are using a pipette you will notice that, as you keep exerting pressure the piston keeps on going down until it meets a initial resistance which we call as first stop. Once it has reached there you can actually exert a bit more force and go down until it reaches a second resistance which we call a second stock. So, this is useful when we are dispensing liquids. For collecting liquids what you should do is, you should press down the plunger or the piston until it reaches the first stop; hold it there and then you dip it into your tube.

So, I want to collect this liquid, I am going down pressing down until I reach the first stock and then what you should do is don t dip it deeply into the liquid do not go in so, deep, that is not good practice. Do not do it too much of the surface because they you take the risk of collecting air bubbles which will put errors in the volume that you are calculating. So, a good idea is to just dip it maybe half a centimeter inside the liquid and then gently, but uniformly release the pressure of your thumb on the piston. As you keep releasing the pressure, the piston keeps moving up and you are actually collecting the desired volume that you have calibrated the instrument to collect.

(Refer Slide Time: 14:56)

So, I have set it at 1000 and what I have here is 1000 micro liters of this particular solution which I am now going to dispense into the tube where I want it in this case you take it you put it in your tube you gently press back until you reach first stock, then you go in for the second stock. You exert a bit more pressure, you go in for the second stock hold for 1 or 2 seconds and then you take out your tip and now we have achieved what we went out to do.

We now have 1 ml of this solution in this container using this pipette. Another thing is after you are finished using your pipette suppose you had used your pipette at 800 micro liters, you needed 800 micro liter of the solution and you collected it do not leave it like this, because that will damage the internal spring mechanism that is present in this instrument.

(Refer Slide Time: 15:58)

When you are done working with the pipette, always keep it in its released condition. The released condition corresponds to the maximum volume that can be pipe it pipette, it out with that instrument.

In this case 1000 micrometer is the maximum volume that we can collect, always release it or keep it when not in use at 1000 micro liters. Any other volume that you keep it at will cause undue compression on the internal mechanisms and damage your instrument. This is applicable for all the different pipettes here for 1 to 10 it will be kept at 10 micro liters and not at use, for 10 to 100 you keep it at 100 micro liters and not at use and so, on.

(Refer Slide Time: 16:56)

So, I have been talking about collecting 1 ml. Suppose I want to collect 5 ml or say 10 ml of a particular solution in which case instead of using this 10 times I would like to use this once. This pipette controller does not use these sort of tips, instead they use serological pipettes. So, this is present in a sterile format its present in a sealed condition once we break the seal we have to use a (Refer Time: 17:22). So, I am going to just take out the serological pipette fix it to this and remove it and this is what we are going to use. You will notice that this can collect a volume of up to 10 milliliters so, let us do that.

The way this operates is you will notice that this is two buttons; the upper button when you press it will withdraw fluid when you stop it will hold it in that particular volume and when you press the lower volume lower button it is going to dispense the liquid let me demonstrate. So, I will dip it into this solution and when I am pressing the upper button see, I am actually collecting liquid from here. Say I want around 10 ml I just take it here and when I want to dispense it, I put it into this tube and now I will press the lower button once I press it, it gets dispensed.

So, yeah if you press it hard it gets ejected rapidly if you press it slowly it gets let me show it again. I am collecting the liquid oh see I was talking about an error right if I do not dip it properly, you saw what happened air bubbles entered the serological pipette. So, I do not want that. So, dip it in nicely perhaps I am sure it is visible now. Dip it in nicely and you go at it at an uniform pace, you are collecting the liquid and dispense

slowly you can dispense drop by drop or if I increase the pressure I can dispense of the liquid as a jet.

So, this is how we use pipette controllers to pipe it out comparatively larger volumes of liquids. In this case you do not have to worry about releasing the people with because this does not work on that mechanism, but in these cases even for multi channel pipettes and single channel pipettes, you always need to keep the pipette in the released condition when not in use.

So, I just showed you how to fix a pipette tip to the pipette. After using it we need to detach this which we call as ejecting the pipette tip away from the pipette it can be done very easily. You will notice that this region slides when I apply pressure, and as I apply pressure this moves down when I apply sufficient pressure the pipette tip falls off that is how we eject tips from pipettes.

(Refer Slide Time: 20:09)

I was also talking about the multi-channel pipette. Remember which can use this one can use eight tips at a single go. Suppose I want to collect 100 micro liters, 8 times instead of doing it 8 times, I can do it in one go in which case you align the nozzles with the tips you press down slight motion and see you actually have volumes here.

So, the idea is I do not have anything to show you right now.

(Refer Slide Time: 20:50)

But what happens is in many aspects of molecular biology or biochemistry you will be using 96 well plates. Suppose you have to collect a particular solution from here in equal volume. At that time you dip it in here I know its empty, but I am just showing you the utility, you dip it in here, you collect the liquid and then you dispense it somewhere else and once its done you eject all the tips simultaneously.

Hello I am Snigdha Maiti I am a Senior Research fellow of School of Bioscience, I am working under the supervision of Dr. Shamode and one of the designated TA for the course of NPTEL experimental Biochemistry, hopefully this course will help you in your research and we could help you.

Thank you.

(Refer Slide Time: 21:39)

Hello today I will show you how to use a pH meter and how to measure on the pH of a solution. So, for that we have a pH meter here and in this monitor you can see the pH reading also the temperature and the volt. And along with the monitor, we will have a electrode which have to be cleaned before we start using it.

So, for that we will remove the electrode from the storing solution which is provided by the manufacturer.

And we will clean it with de ionized water rinse it, and then with the help of a tissue paper we will wipe it down so, that the electrode is dry.

(Refer Slide Time: 22:25)

So, now theat I have already mentioned you about the pH meter. So, this is a digital pH meter and the probe should be always kept inside the solution storing solution.

So, basically the storing solution will be provided by the manufacturer, different pH meter companies of pH meter they are everyone provide the storing solution. And this is a solution of three molar or four molar potassium chloride solution. So, keep it in mind that the probe should be always moist, otherwise if it is it becomes dry it will damage the probe and you will not get the accurate result.

. So, from the storing solution this big container, I have taken out small volume of the storing solution and kept it in a small container. And for storing this probe for long time I have kept the dip the probe inside the storing solution. Now before you may start measuring a pH of a particular solution or a experiment, we you should always need to know if the pH meter is properly calibrated or not. At a regular interval all the instruments should be cleaned and calibrated otherwise accurate pH will not be showned.

So, for that today right now I will be showing, how to calibrate your pH meter using three different solution and this is known as the three point calibration method.

So, for that again the manufacturers have provided us the different solution, one is of page 4.01 0 1 another one is pH 7 and the other one is 10.01..

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So, all these solutions are basically buffered and from these three bottles I have taken out or allocated small volume of these solutions into three falcon tubes.

So, now I will be showing how using these three solutions will be calibrating our pH meter.

(Refer Slide Time: 24:18)

So, in order to do the pH meter calibration, first I will take out the probe from the storing solution and as I mentioned earlier, before you dip the probe in any solution you have to

wash thoroughly with de ionized water. Now while washing do not damage the probe always use de ionized water.

So, after you wash the probe, then you dab it with a clean tissue paper do not rub the probe otherwise you might damage the probe and it is very sensitive and fragile and then you open this small cap. So, this is a refillable probe while inside this probe, there is a an electrolyte reference electrolyte and before you start measuring any pH you should open this now.

(Refer Slide Time: 25:13)

Now I am using a standard solution of page 4.0 and then I will dip this probe inside this solution. Now be very careful how much you dip the probe inside the solution. The probe should be or the bulb should be absolutely immerse inside the solution do not drip it till here otherwise this buffer might go inside your probe.

So, dip it until the probe is fully immersed inside the solution. And then in this monitor, we will put there is a calibration setup and we will press the calibration setup and now it will show one point. So, if you then start it will start getting the pH of that particular solution. Now you have to wait for some time before this reading becomes stabilized; do not rush here otherwise it might be inaccurate. So, this is blinking right now until analysis stabilizes wait till that.