**Interactomics: Basics and Applications**

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**Lecture – 42**
**Label-free Quantification of Proteins using BLI**

As we have been discussing about different type of biosensors. We discussed about SPR and now we are discussing about BLI or Bio Layer Interferometry. In the last lecture you got a glimpse of how BLI can be used to perform the kinetic analysis of protein protein interaction. Today we will be focusing on another application of biosensor that is biomolecular concentration analysis. The task of measuring the concentration of a specific protein in a complex solution is not simple.

Therefore, a reliable analysis method for the same is desired which enables the estimation of biomolecule concentration in the complex sample matrix. In this concentration assay, different concentration of the analytes are injected and the response from these different concentrations is plotted against the concentration of each sample. From this, a calibration curve is calculated which is then used to determine the concentration of the same biomolecule in the sample matrix.

Today we will be studying the concentration analysis of a protein apolipoprotein e by plotting the standard curve through bio layer interferometry based experiment. Let us welcome Mr. Susheel Vaidya for today's lecture and demonstration session.

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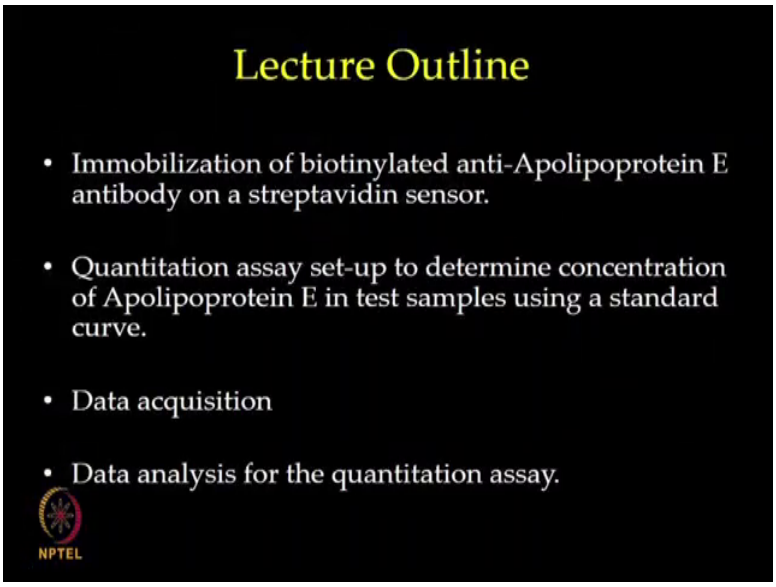


Good morning one and all, myself Susheelendra Vaidya from pall corporation. I am going to give a demonstration on how this BLI Technology we can use in the quantitation. Now, I am going to demonstrate the experiment here is like that quantitation of the apolipoprotein using a antibody against the apolipoprotein.

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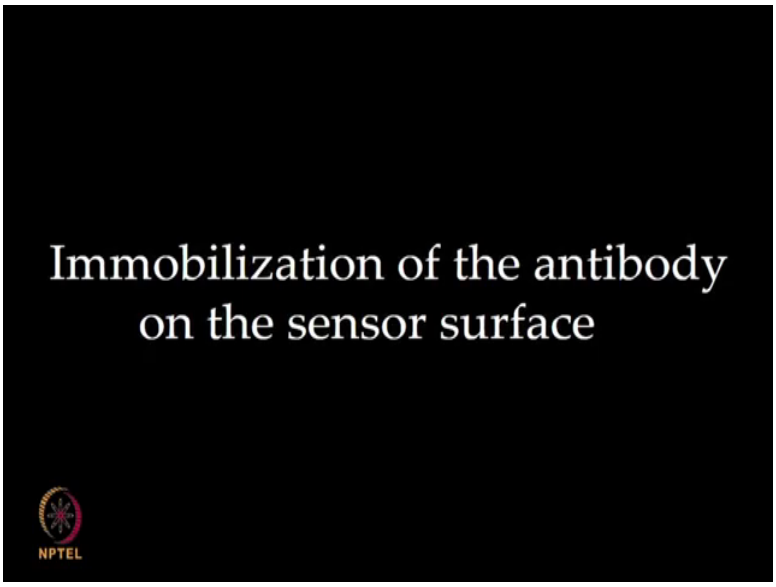

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The biotinylated antibody I am going to use immobilization of the antibody on to a streptavidin sensor. This is the streptavidin sensor I am using for the immobilization step.

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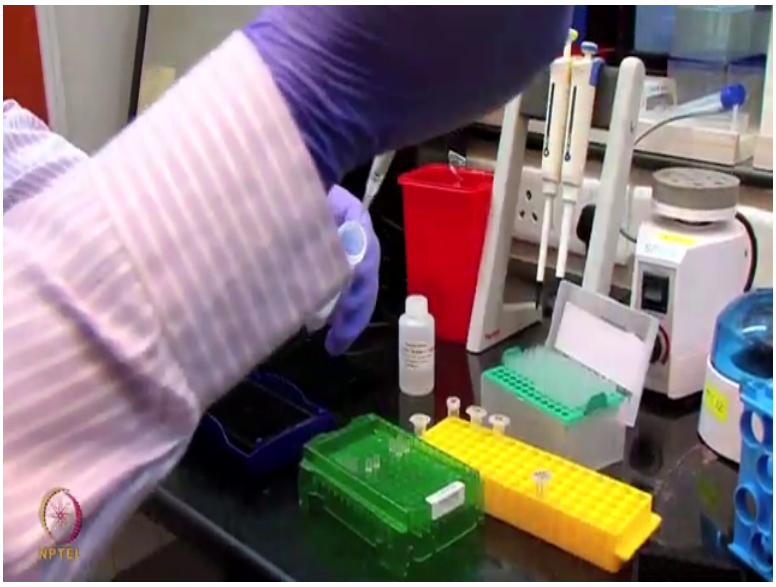

So, here in this experiment we are using a streptavidin sensor the streptavidin sensor has to be hydrate behalf of the immobilizations. Now, I am going to do this hydration of the streptavidin sensors. What I am doing is, I am adding the 1X PBS into the 96 well plate, 200 micro meter of the 1X PBS well. So, I am going to here.

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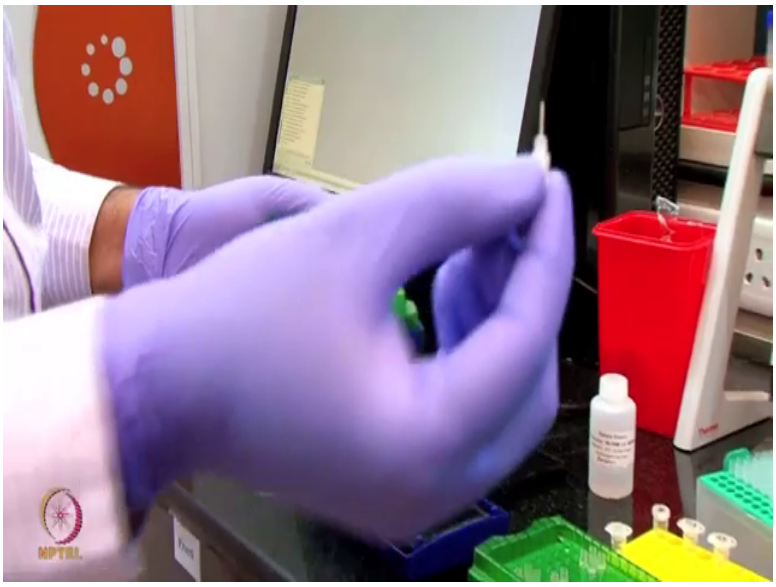

Now what I am doing is, next step; I am taking the bio sensors which is the streptavidin sensors, these are the sensors looks like this. I am going to put on to the sensor tray, this is the sensor holder tray I am putting this sensors like this. Now this sensor tray I am keeping for the hydration for the 10 minutes. So, this is I am going to keep like this. So, now, I will set aside this sensors for the 10 minutes.

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Now, I am going to demonstrate you that how we are going to immobilize the apolipoprotein anti apolipoprotein on to the streptavidin sensor. I am taking a 200 micro litre of the 1X PBS buffer, this is pipette. I am going to add 200 micro litre each to a well 96 well. Now I am putting the this is the biotinylated anti apolipoprotein.

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So, now I am going to add 200 micro litre of each biotinylated anti apolipoprotein to a 96 well plate.

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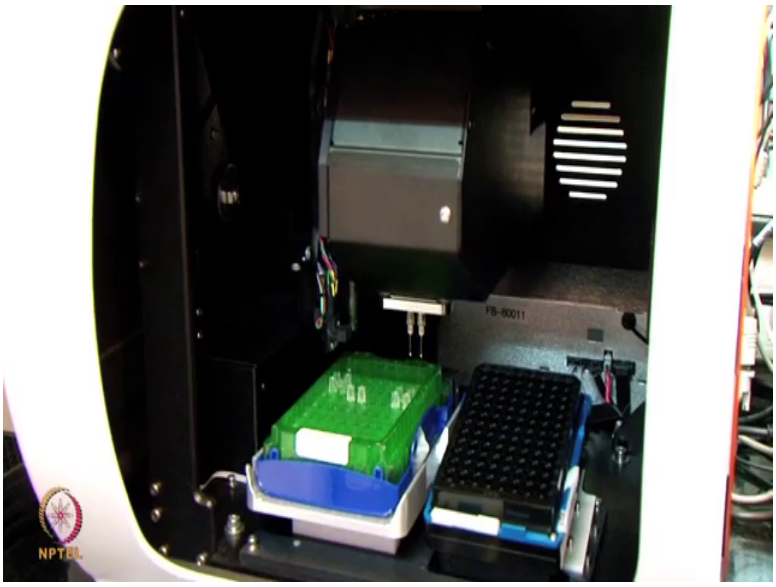

Now, this now the sensors are hydrated, I am going to place in the instrument the sensor holder, I am placing here like this. It should be firmly fixed in the group and now I am going to put the sample plate which contains the biotinylated antibody I am going to place like this in the sample plate. This is we call it as this particular part we call it as a sample compartment, this part we call it as a sensor compartments.

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Now, I am going to click on to the data acquisition mode. So, when we click on the data acquisition the instrument.

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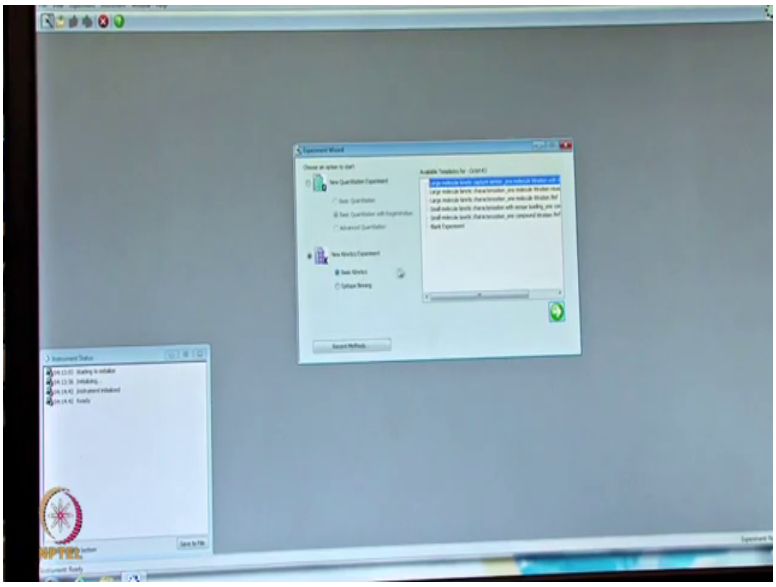

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Now, we can see this how the movement of the optical head can frequencies of this spectrophotometer itself is initializing in the instrument. In the monitor during the initialization we can observe this initialization status once it is comes the signal as a ready then we can use for the immobilization step. So, now, you can able to see here the instrument status it is ready when we click on the experimental wizard setup you can see that there is a new quantitation experiment as well as the kinetics experiment. Here I am doing the immobilization step. So, that is why I am choosing the basic kinetics with the experiments.

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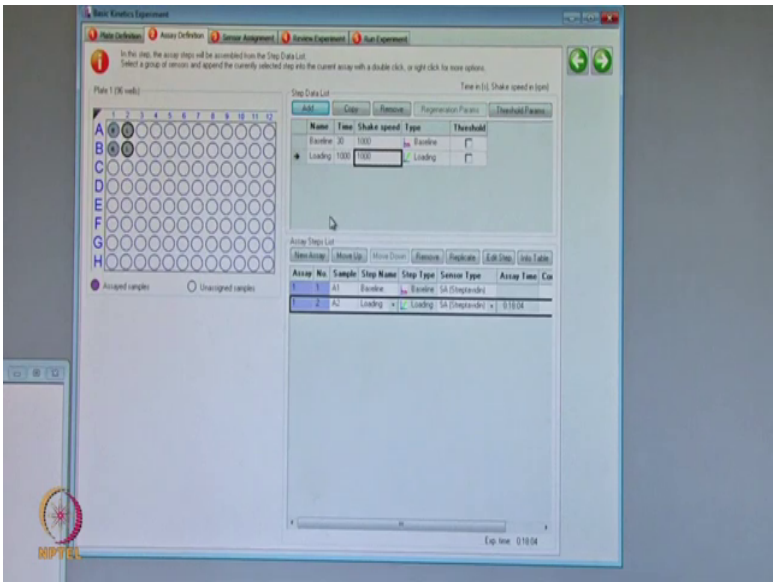

I am choosing this blank experiment, then I will say yes go. Now you can see there is a page. In this page its on your left hand side it is showing that 96 well plate. What things we had put it in the 96 well plate? Here I had put in the in the a one and the b one here I have put a buffer right, we call it as a buffer, right click it and save it as a buffer it is indicated by A B, then we had to select these two.

Now, we have to mention this step as a load. Load is nothing but the immobilization of the biotinylated antibody against the apolipoprotein that we had placed in the second A 2 and the B 2. So, we have to mention here in the sample id just it is a buffer ok, then quite copy. Now it is biotinylated ap apo I had mentioned biotinylated apo. And I can copy it and paste it here in the next step in the assay definition we have to mention what is the steps the instrument has to be perform.

The first step is baseline and just a second step on clicking onto the add here we had to load. The next step is loading step nothing but, your immobilization step, I have to say ok. Now, first step is baseline, I had to take a arrow mark here right click here in the next step is the immobilization we have to use the loading, this is the time here it is mentioned as 30 seconds I have mentioned here around 1000 seconds for the immobilization of the antibody. I am using the streptavidin sensor by default it is coming as a streptavidin this is we had assigned the assay definition step this sensor assignment.

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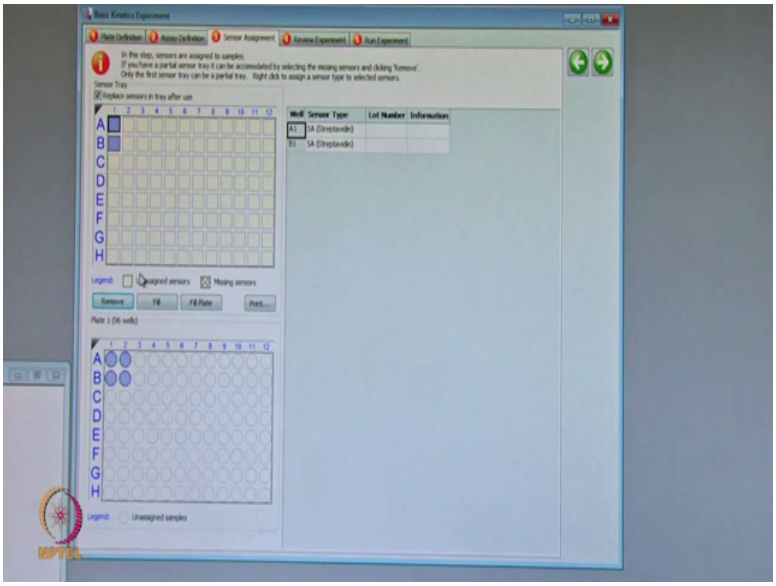

Where you had kept the sensor? In the sensor compartment we had kept a sensors is a A 1 and the B 1and it is by default it is showing.

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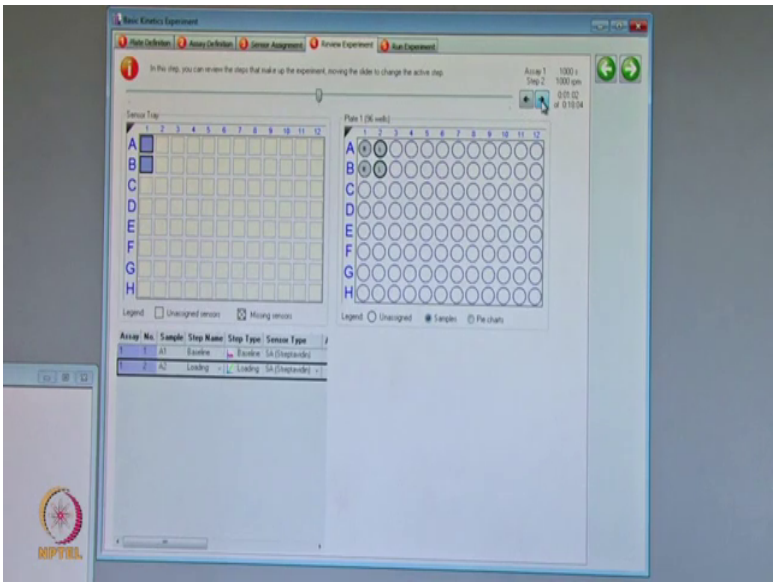

If it is somewhere it is in the tray we have to assign that. In the review experiment whether my methods are fine or not we can we can test it. Thus first step is the sensor has to be picked from the A 1 and the B 1, it is going to the baseline that is nothing but your buffer it is highlighting by the black colour on the surface.

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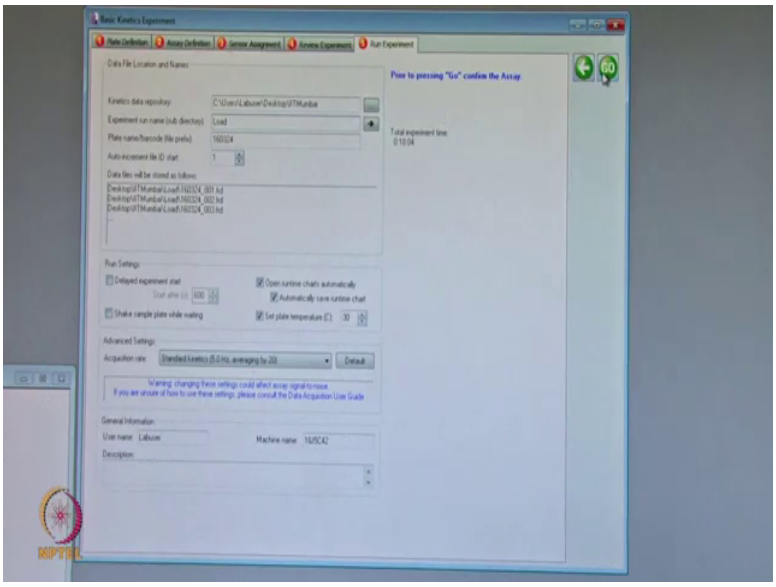

So, next step is your loading step so, it is moved. In the run experiments, where you want to save the data; I will select this and the experiment I think you as a load ok. Then I can uncheck this these twos set go here.

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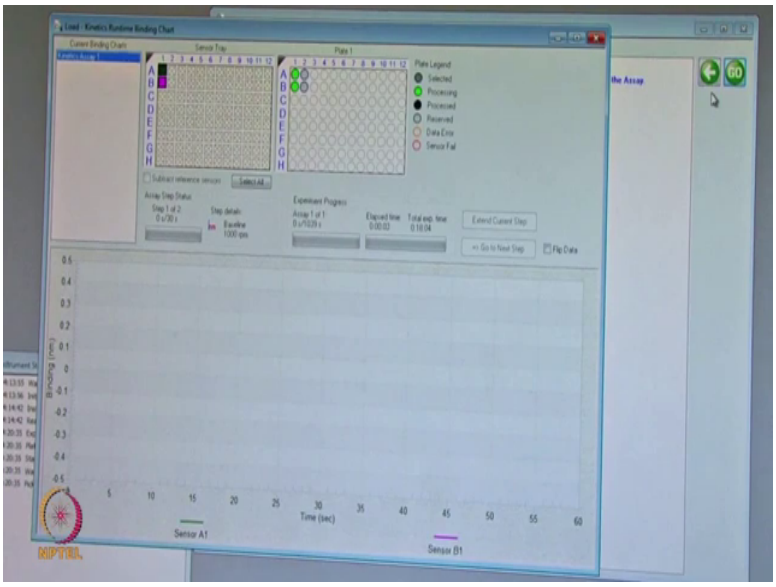


Yes now the sensor is picking from the sensor tray here yeah if you look at there is a shaker here, we are working at the 1000 rpm speed, it is going to the baseline, it is applying for the 30 seconds the baseline. Now, you can see the immobilization step the biotinylated antibodies going to immobilize onto the streptavidin sensor.

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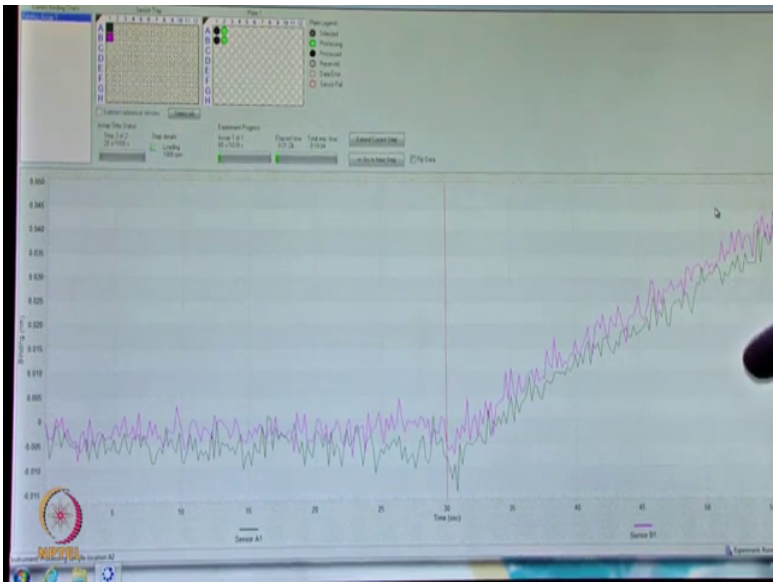

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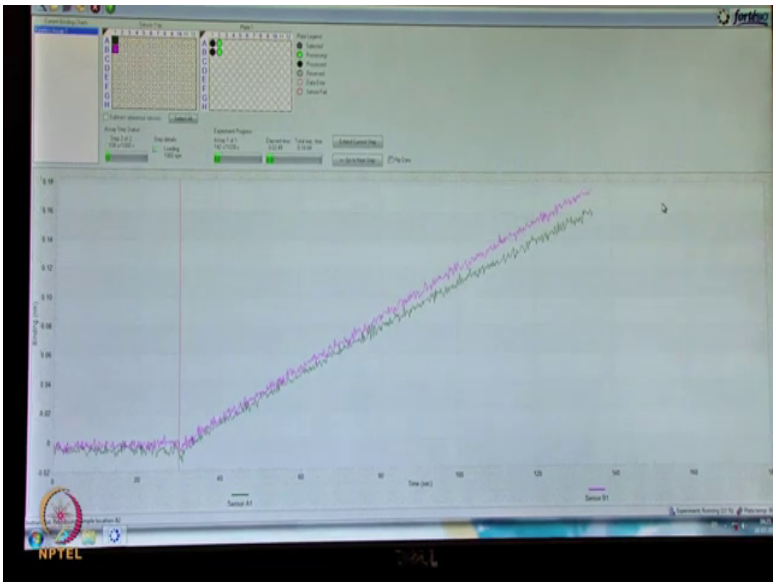

Generally, we will load it has to read the equilibrium roughly around it should be a more than 0.7 nanometre is wooden up for pre immobilization level we can go for the quantitations.

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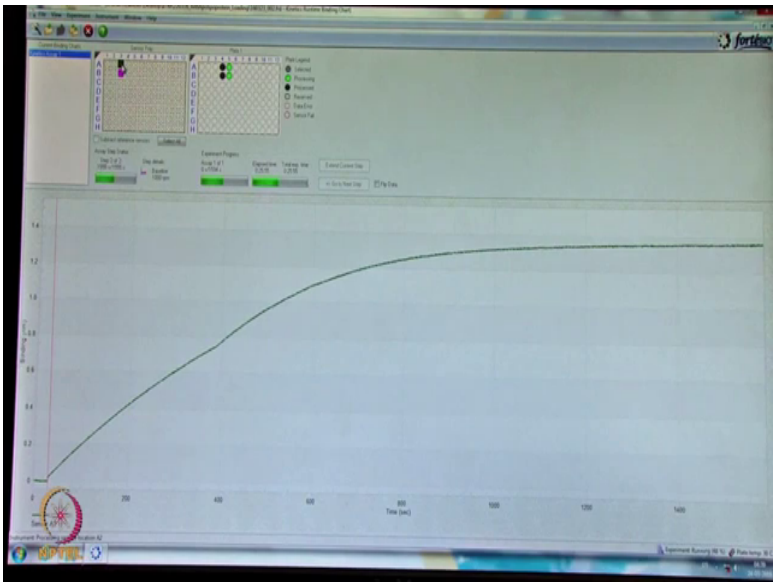

Now, you can see the desired level of the biotinylated antibody immobilized onto the streptavidin sensors. Now we can able to see there is a 1.4 nano meter loaded onto the sensor.

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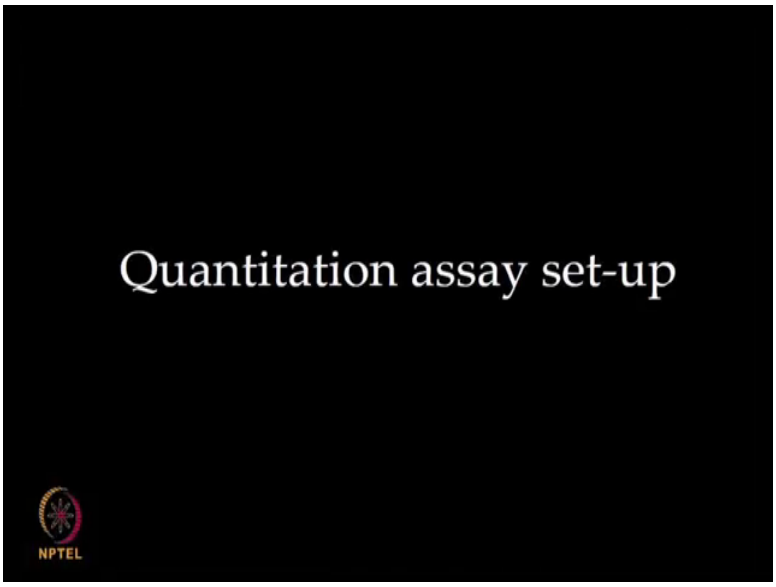

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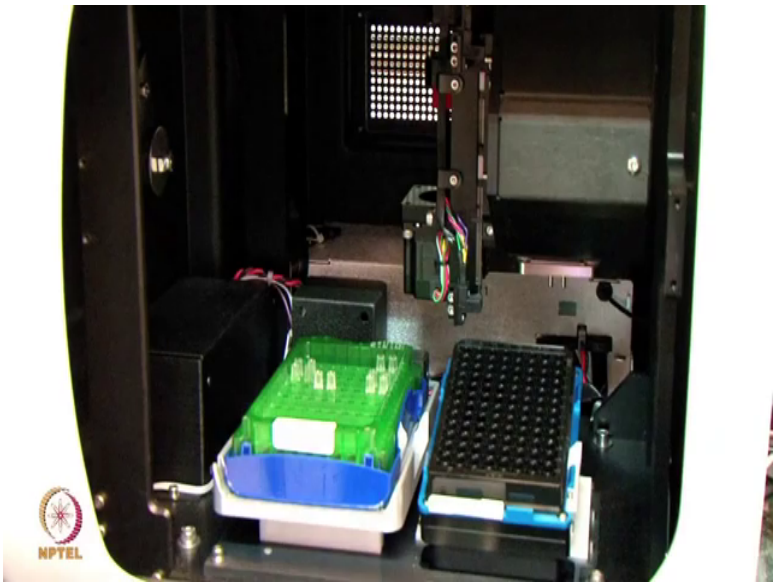

Now, I am going to take back this sample plate and also sensor tray. Now this sensor actually which is vibrated in the PBS buffer and also our biotinylated antibody also it is in the PBS buffer.

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Now, I am going to equilibrate these sensors with the PBST buffer, the PBS buffer having the tween in that.

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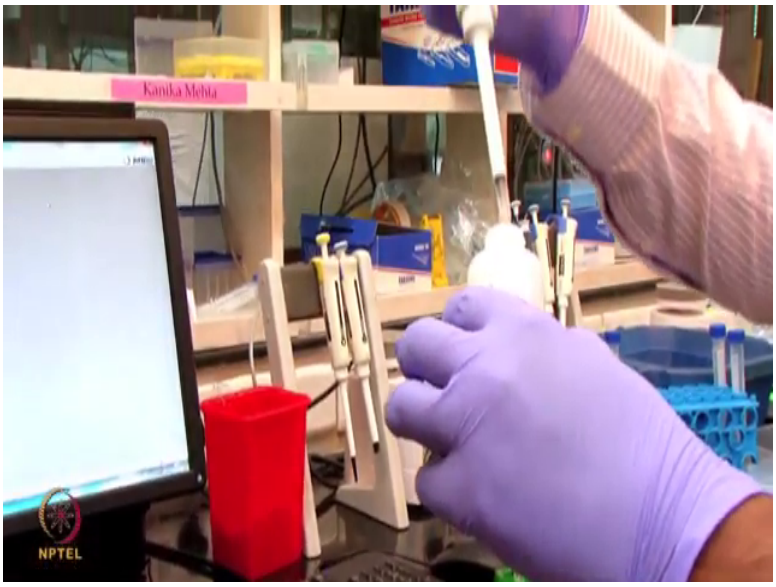


Then this is the PBST buffer, we got it has a sample diluent buffer, here I am going to take a 200 micro drop this PBST buffer I will add here in the two; A 2 and the B 2. So, I will now the sensor position is I am changing from A 1, A1, B1 at like that A2, B2. So, now, I will start to equilibration of the sensor with the this particular buffer.

We are going to do a quantitation of the apolipoprotein. So, the in the previous step; what we have done is we immobilize the biotinylated antibody onto the streptavidin sensor. Now we can do the quantitation using that sensors is pre immobilized. So, what I am going to do is, I am making a apolipoprotein standard with the concentration is 15 microgram per ml.

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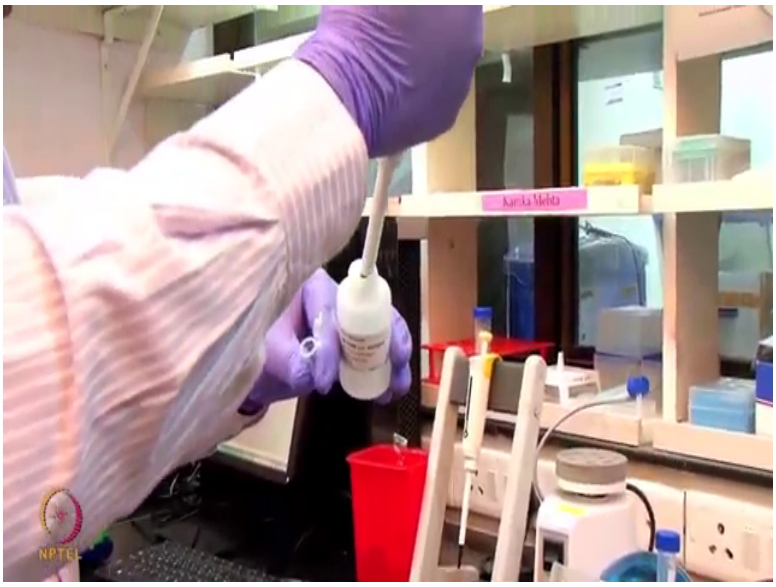

So, I am, here I am having a apolipoprotein standard, I am going to dilute a 50 micro gram per ml using a sample diluent.

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So, from this I am going to do a this standard, I am going to do a serially dilution like two fold dilution I am going to do with the buffer.

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Here to that, what I am doing is; I am taking this 96 well plate I am adding the buffer into the wells here I will add I am going to use their around the 6 concentrations they are two fold dilutions. So, I will add here buffers I am going to do a dilution in the plate itself.

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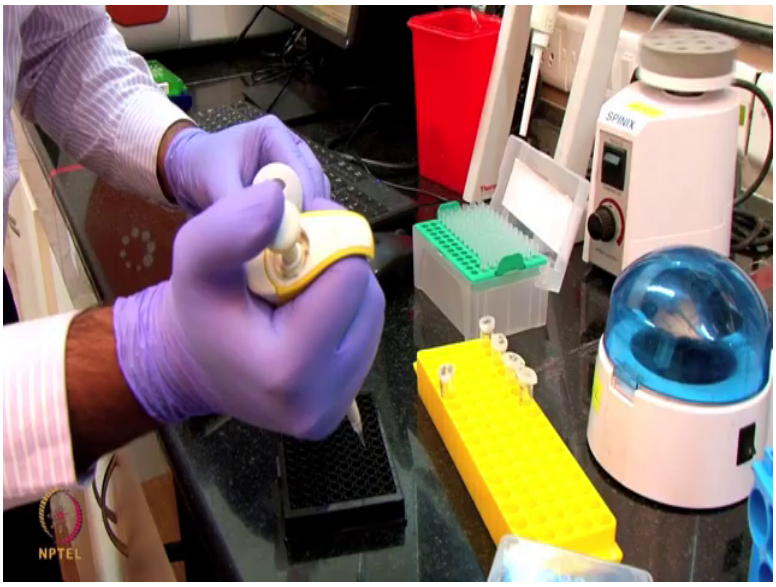


So, 200 micro meter each of these sample diluent buffer I am placing in the 6 wells of the 96 well plate. So, I just and I am going to use here 3 as a references. This plain buffer uses as a reference here, this is the 50 microgram per ml protein I am I am serially diluting I am doing a two fold dilution in the plate. So, now, we had made a 6 concentrations of the standard apolipoprotein. So, starting from concentration range 1.56 to 50 microgram per ml.

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I had put these standards the 1.56 in from A 3 to a till A 8, that is the last 50 microgram per ml as well as I had put a reference buffer the buffer which we were using for the reference subtraction here, so that, I had put it at the B 6, B 7 and the B 8.

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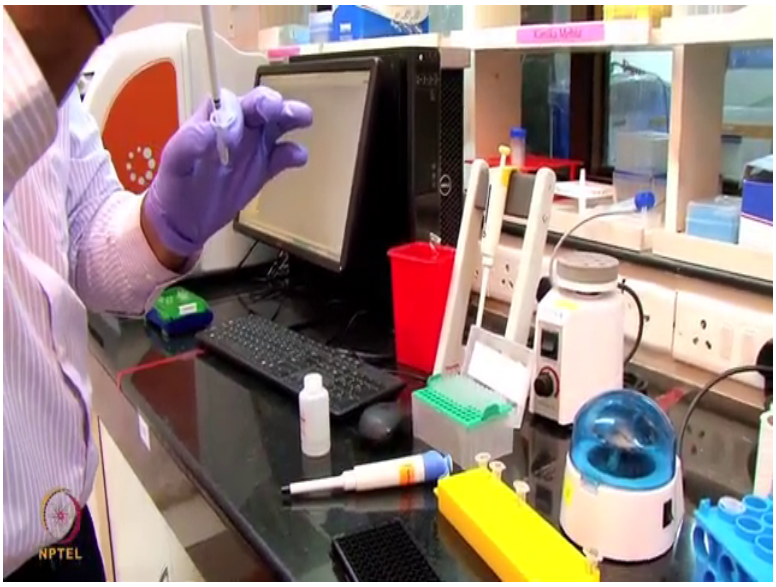


Now I am going to add a unknown samples. We have a 3 unknown samples here. So, that I am going to put it in the B 3 B 4 and the B 5. So, these 3 unknown proteins we are going to determine the concentration of the unknown apolipoprotein in the in the samples using the standard. Now I am going to add the 3 unknown samples here. I am going to place at the B 3, this is the sample number 1, which is sample number at B 4; the sample number 3 at B 5. So, there are three unknown samples.

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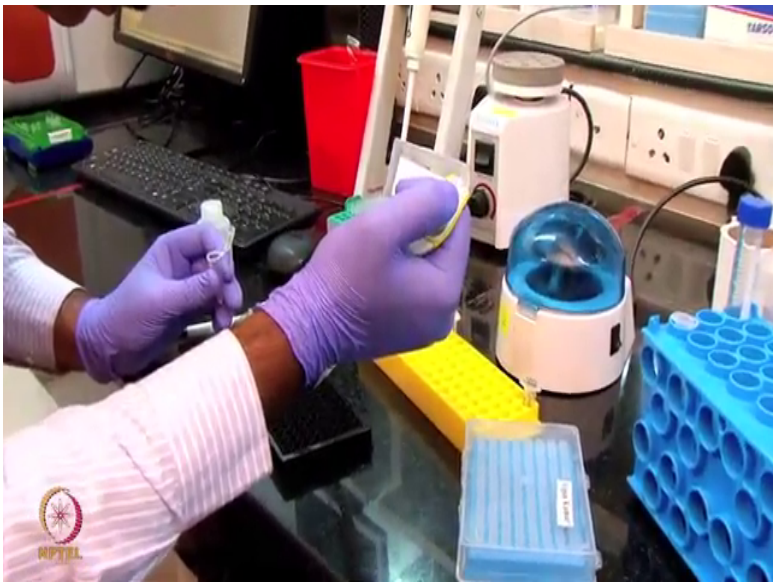

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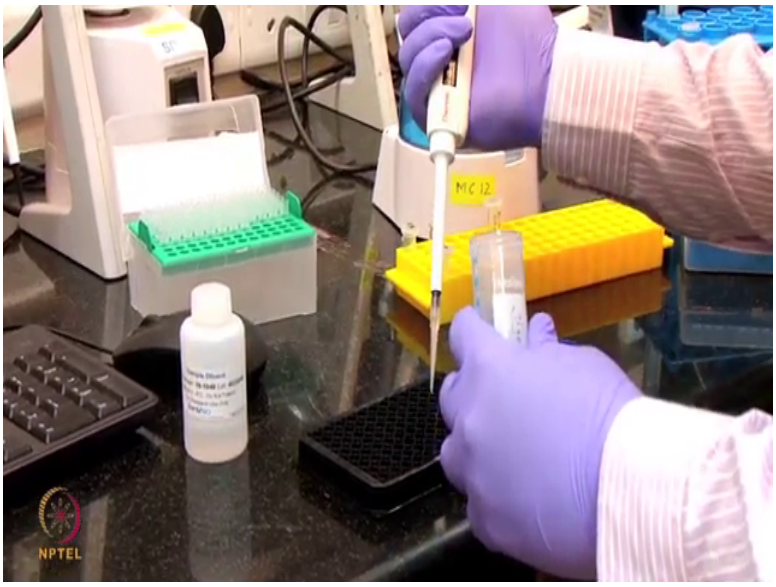


So, I am I am using the glycine buffer that is a ph to 10 millimolar glycine buffer ah. This is used for the regeneration of the sensor.

So, this the regeneration buffer I am going to place at the A 11 and the B 11. So, this is well the bones apolipoprotein will be washed when the this sensor dip into the this acidic buffer. So, also we have to place one more which we call it as a neutral buffer, that is what this PBST buffer. I am going to place this one at the A 12 and the B 12. Now, I am placing this sensor tray into the instrument at the sensor compartment and also we had prepared the sample tray, here we had put the unknowns as well as the standards apolipoprotein in the sample plate.

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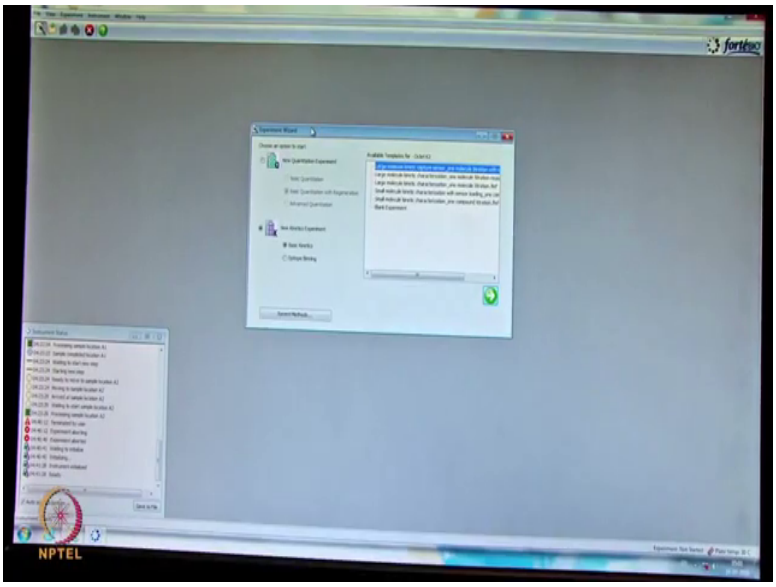


Now, our aim is like here we are performing a quantitation experiments. So, now, we have to choose the quantitation mode here, new quantitation experiment. Here in this we are using a basic quantitation with a regeneration, because two sensors we have immobilized and then same sensor will be regenerates and it can reacquired the different concentration data.

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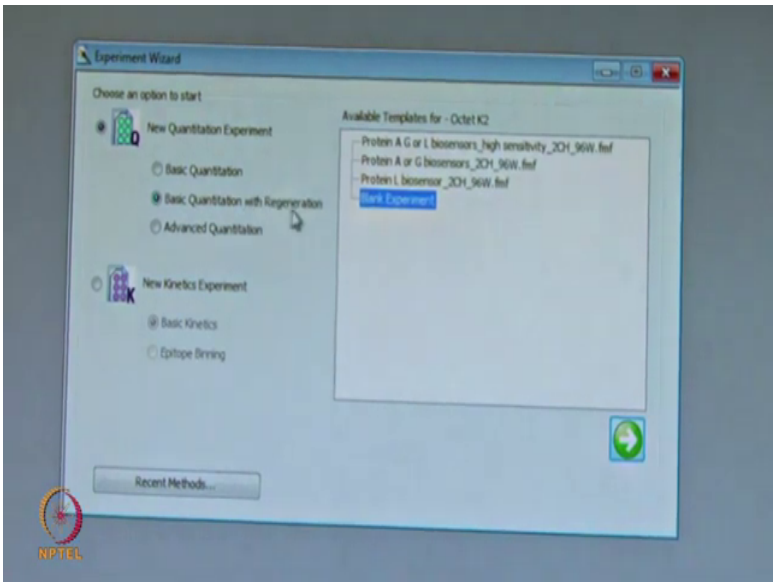


So, we have to chose the blank experiment and say basic quantitation with the regeneration blank experiments then we have to say go here if the experiment. When you open this experiment we will see the plate definition.

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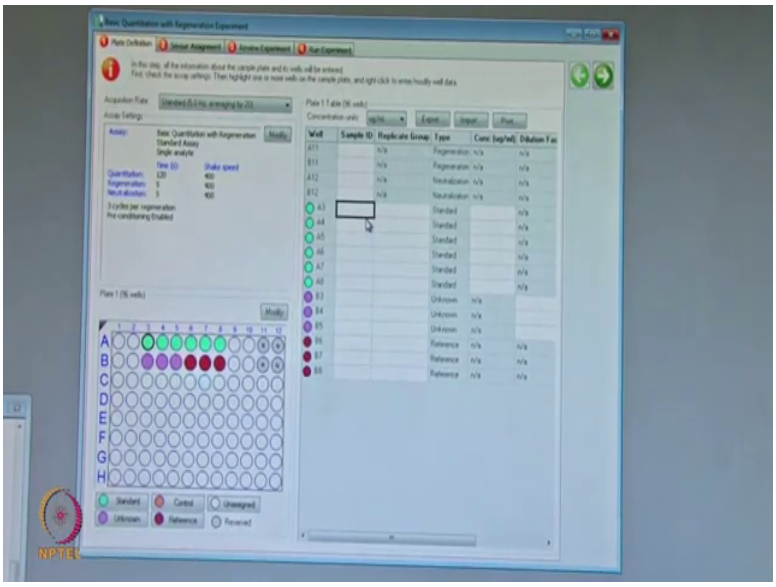

If the plate definitions, what samples we had put it here? So, here from A 3 we had put a around standards A 3 to A 8, 6 concentrations we have chosen. This 6 concentration we have choose it this as a standard ok. Now we can see the this connected as a the green colour and also here one more we had chosen these three are a unknown samples and these three are your reference, we can choose the reference or later also we can we can take it a B reference into a like even unknowns. Later in the data also we can in the that can be modified as a reference.

Here in the table on your right hand side we have to put the information here in the A 3 what we have to put and A 8 what we have to put.

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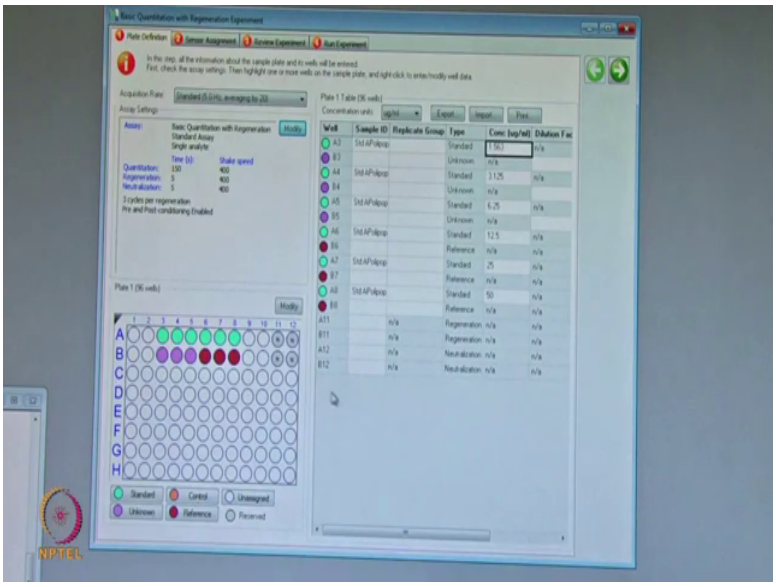

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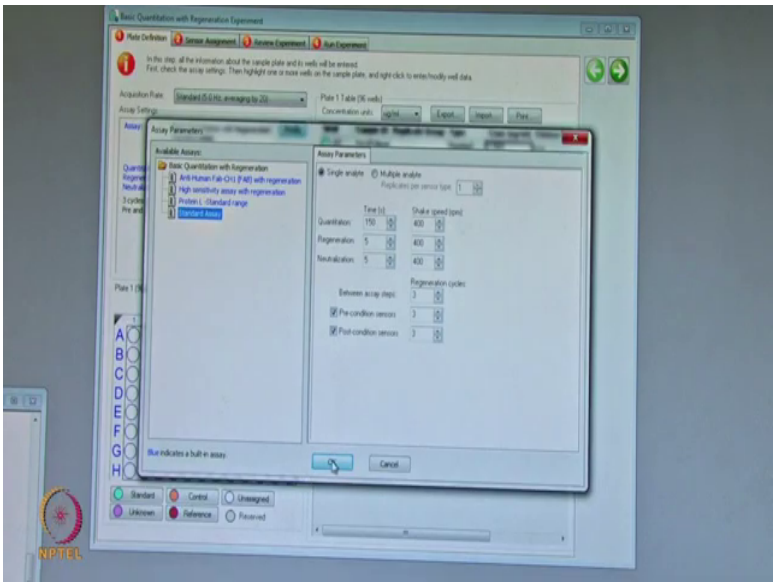

In the method modified section here single analyte we have to choose 120 seconds we have to use a 400, 400 the regeneration is 5 seconds, it will dip in the acidic buffer, 5 seconds it will dip in the neutral buffer. That that cycle has to be perform at least for the 3, 3 cycles.

So, then precondition means; sensor first will regenerate and then goes to sample. Post condition is nothing but, once the sensor is put it into the sample after that it will go for the regeneration. So, we have to choose both pre condition and the post condition sensors. 120 seconds is the acquisition time, I can still extend to a 150 seconds here then say 400 is the shake a speed I will say here. Then in the sensor assignment which is showing so many sensors here. But we are using a two set of sensors for the acquiring the all the samples.

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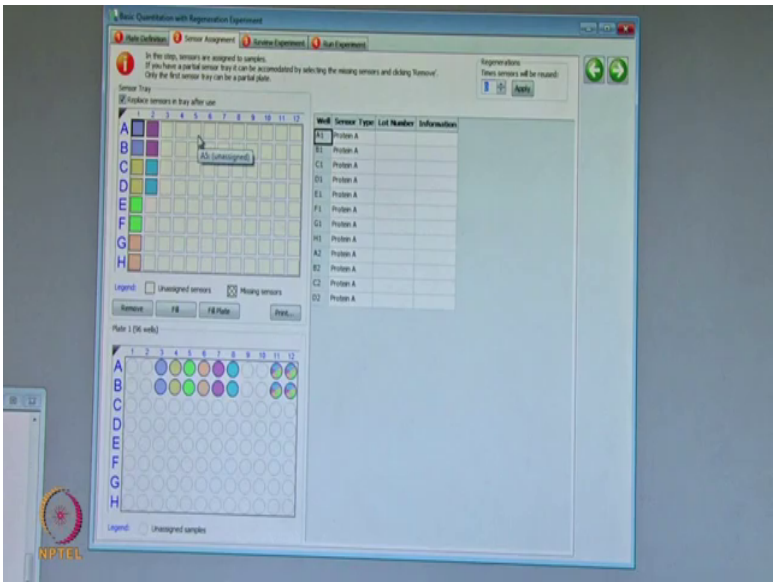

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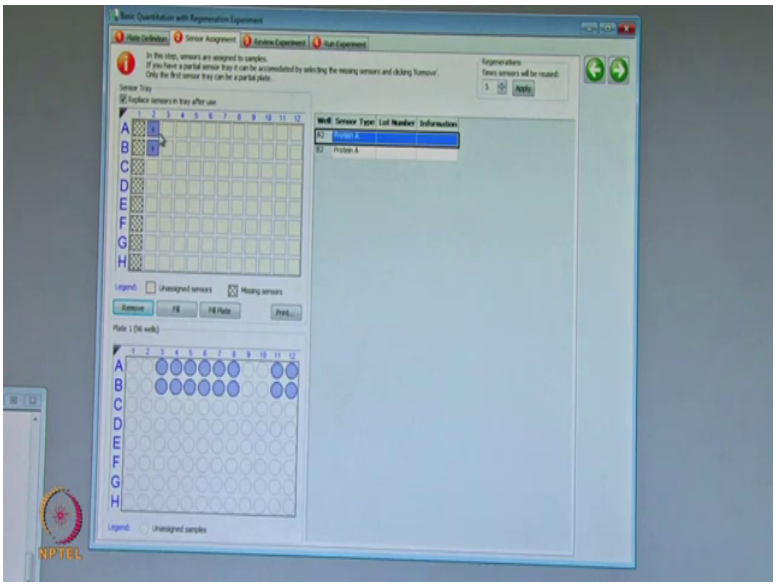

So, now we had kept a sensor at the position 1. So, I will say a remove automatically your sensor is placed at the second. We had kept sensor for the equilibration using the PBST buffer.

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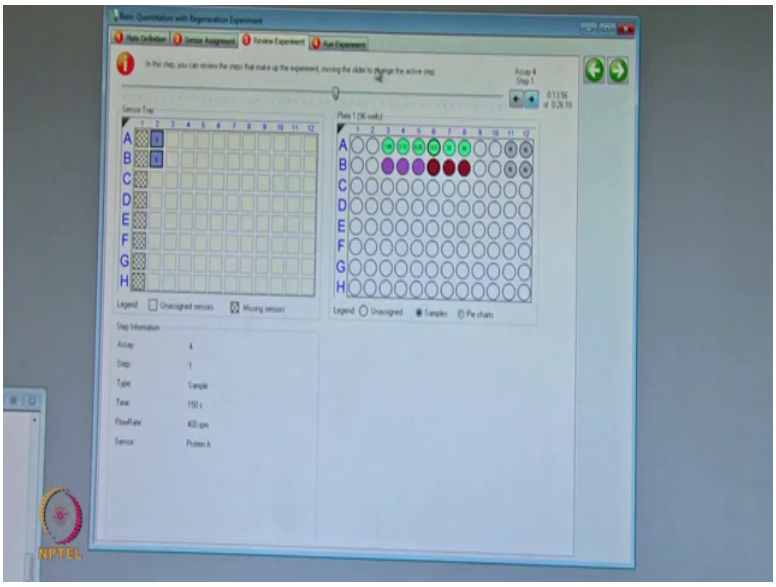

In the review, we can see how exactly the instrument will perform. Sensor is picking from the second position it is going to a regeneration then if you click on this arrow it is a neutralization, regeneration, neutralization, regeneration, neutralization.

In the method we had said that it has to in the plate definition, we had mentioned that it has to perform for a 3 cycles. So, that is what it is indicated by in the experiment it is acquiring for a 3 cycles. Then, later it goes to a sample then once again regeneration neutralization regeneration neutralization regeneration neutralization then goes to here second step and regeneration, neutralization, regeneration, neutralization, regeneration, neutralization. And the third same the process we will continue till the last.

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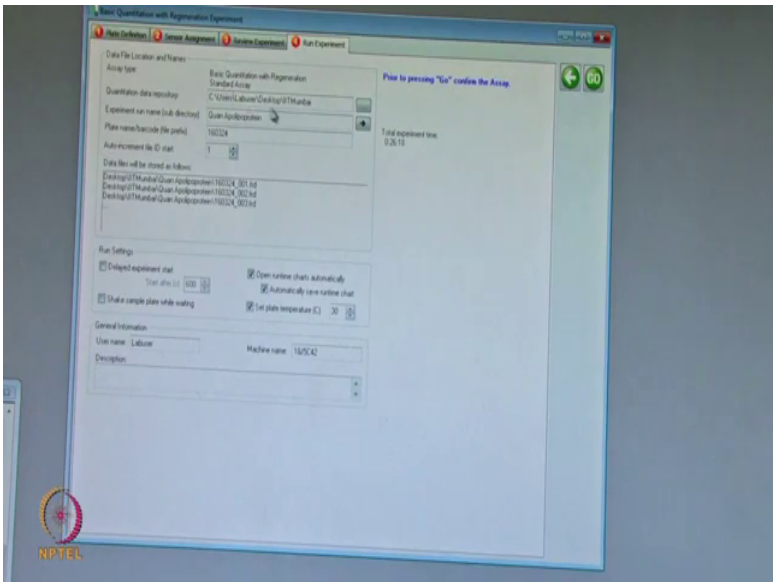

In the run experiment; where you want to save the data? I will mentioned this file as the Quan Apolipoprotein ok. Sensors already higher related I will uncheck this then I can say go here.

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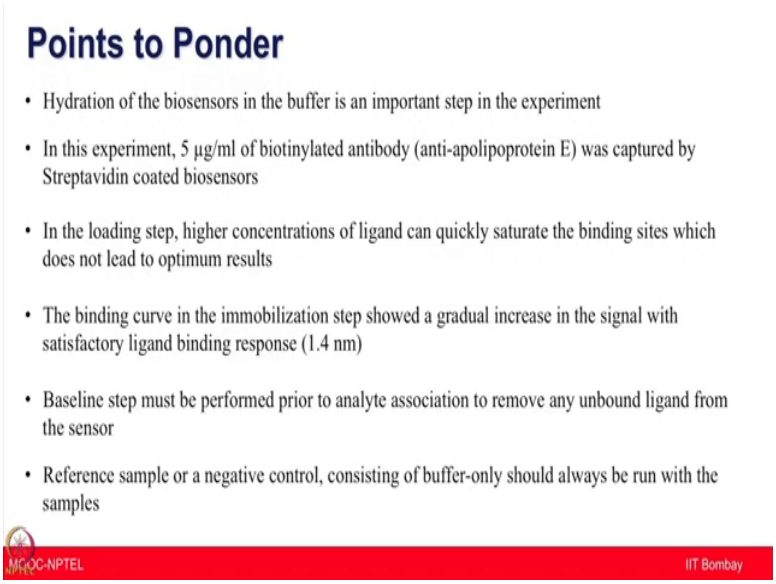

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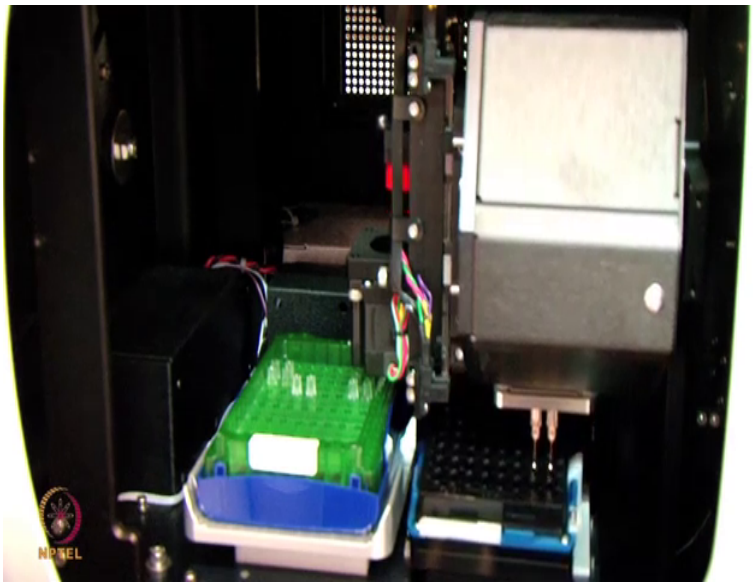

Now, the instrument is picking the sensor and this is a Watt and it is going to a regeneration.

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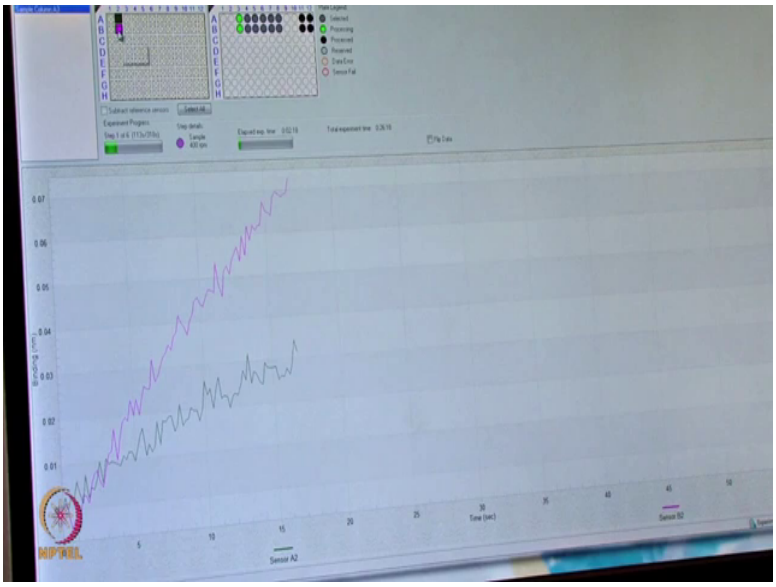

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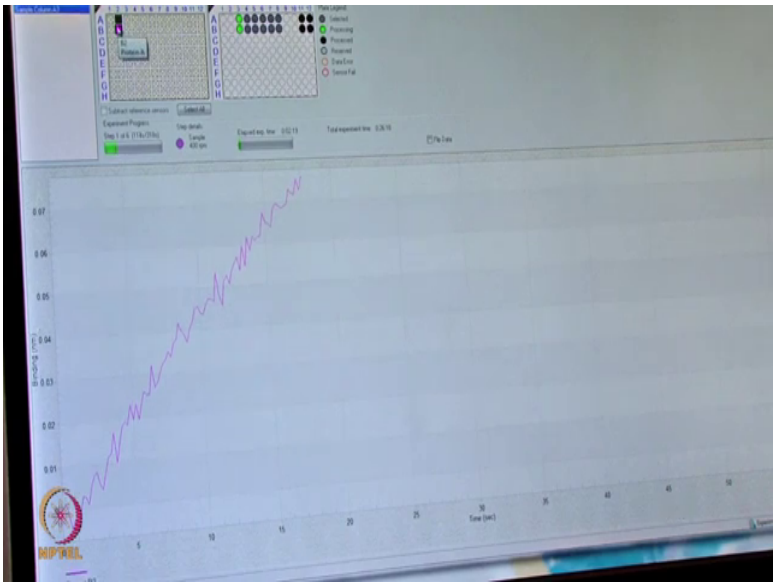
0.07
0.06
0.05
0.04
0.03
0.02
0.01
5
10
15
20
25
30
35
40
45
50
Time (sec)
NPTEL

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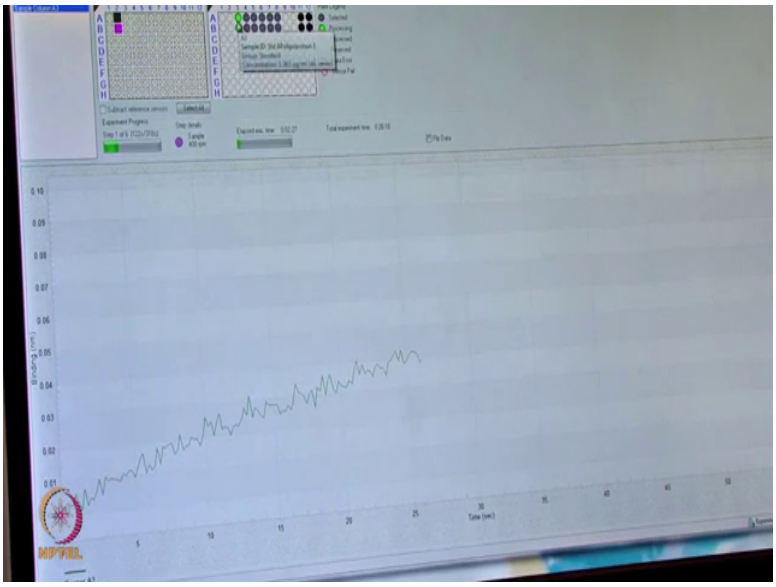

See the pink one is the one which is unknown sample here and the green; the sensor it is indicating the lowest concentration which is around 1.5 microgram per ml.

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Once the data is acquired, the green indicates this is the standard and as well as the pink indicates this as a unknown sample.

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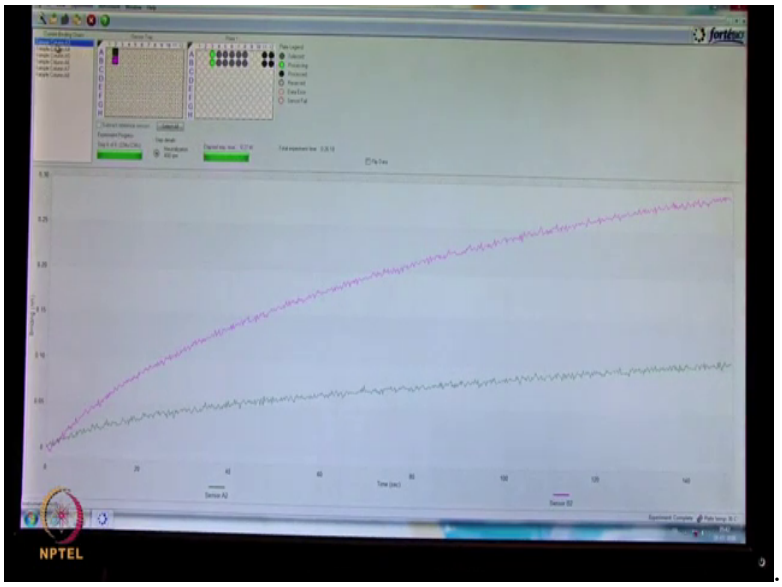

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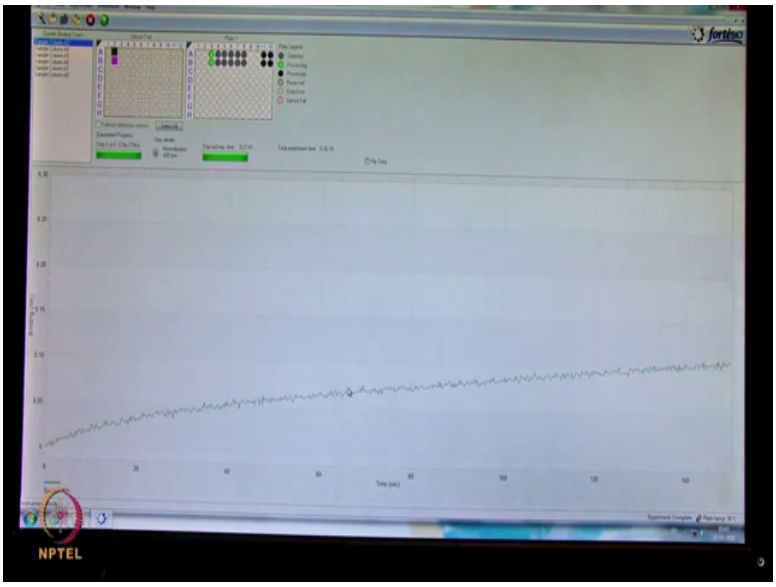

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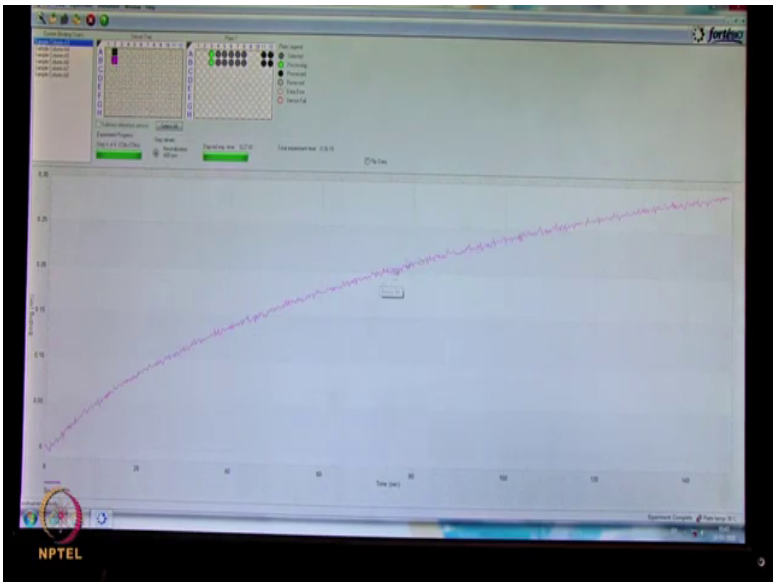

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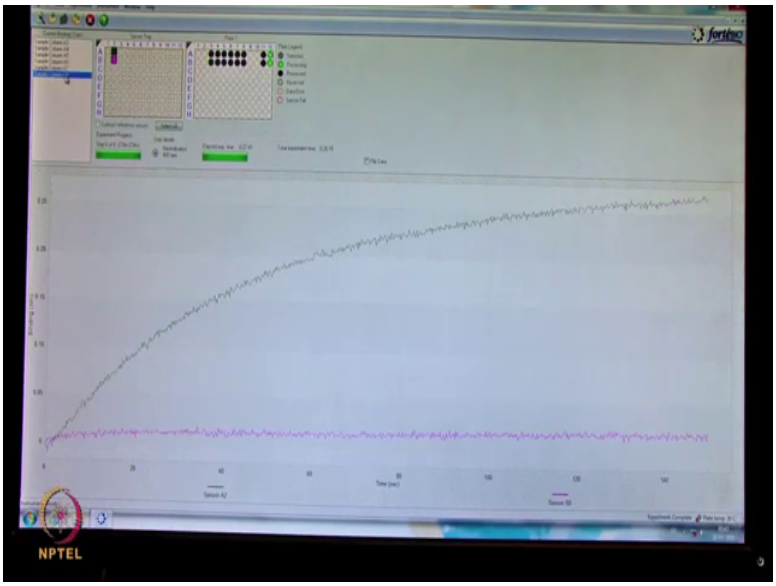


Now, the acquisition is done we will go for the data analysis.

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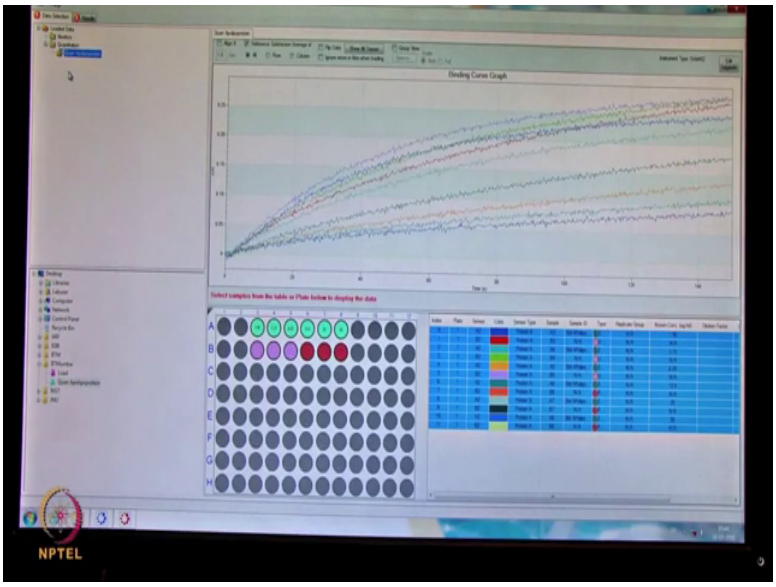


This is the file, this is the acquisition file indicated by a green colour here. So, now we can see this the graph, it is indicating the all your standard this is your standard graph the one which is the 50 microgram is the highest the binding curve is showing.

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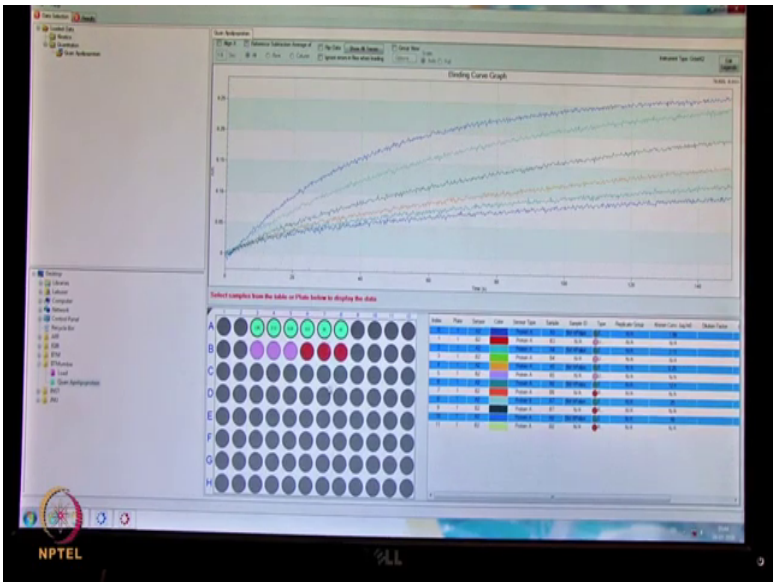


And the then the second, then the third, then the fourth and the fifth concentration and the sixth. So, highest will be the 50 and the lowest will be the 1.56 microgram per ml.

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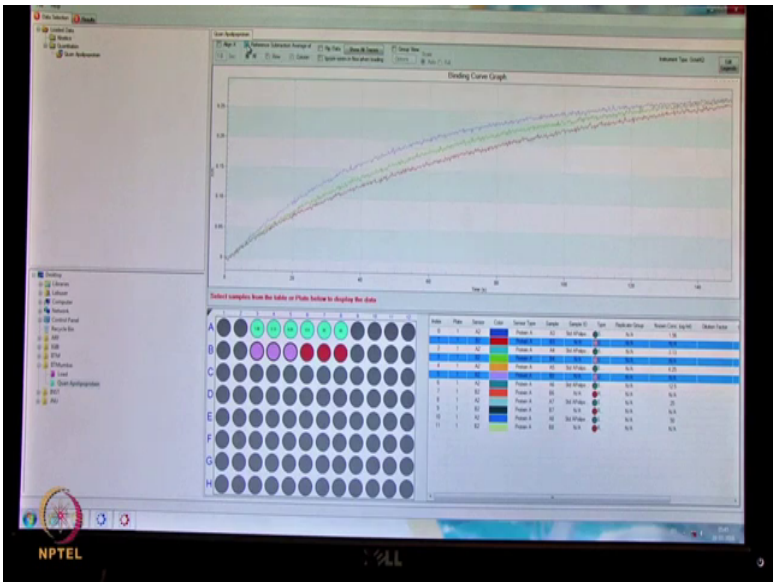


Here there are the three unknown samples also we had kept in the in the B 3. B 4 and the B 5 and the three reference samples these reference samples are nothing but, your buffers. Buffers also sometimes gives the artifacts to subtract that the artifacts from the signal we are we have to use a method called as a reference subtraction method. Here we are going to click this reference subtraction average because these three are wells out the buffers. So, it will get subtract from the all the acquisitions for a different concentrations as well as the unknown from that.

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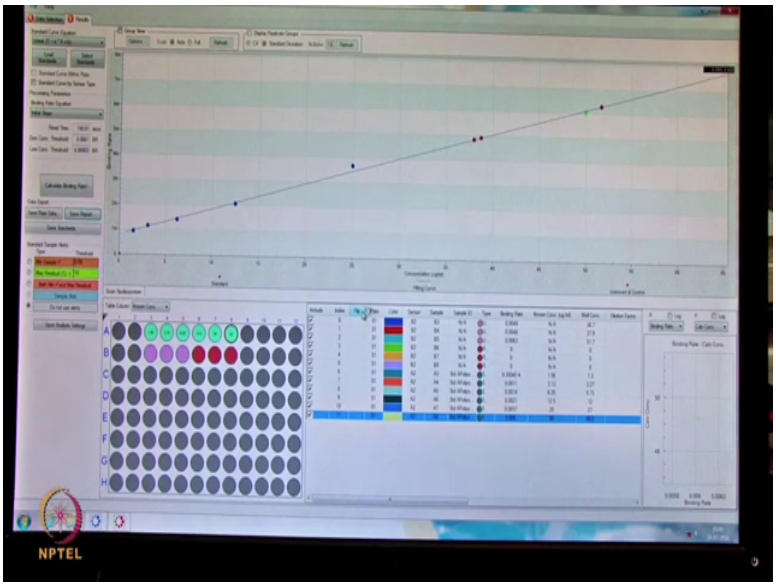


So, now we have to go here in the result section in the result sections we have a different equations here in the standard curve equations which one you want to choose like linear point to point we have 4 pl methods, 5 pl methods as well as linear y is equal to A M X plus C kind of equation A into X plus B.

So, we will go with this equation, linear equation here and also we had put standard in the plate itself as well as the unknowns. So, we can easily do a data analysis we can quantitate the unknown samples. Then we say calculate binding rate. When you say calculate binding rate, we will see the straight line. The blue dots indicates the different concentrations of your standards and the red ones which indicates your unknown sample. There are the three unknown samples here.

So, in the unknown sample we can see a standards if you look at the standard 50 microgram, the back calculated instrument back calculated it recovered as a 49.2 percent, if you see the percent residues that is what we are saying it should be the plus or minus 10 percent for the acceptance criteria. So, this is we are getting around 49.2, 25 we are getting as a 27, 12.5 we are getting as a 12, 6.25 we are getting as a 5.73, 3.13 we are getting around 3.27 and 1.56 we are getting 1.3.

But somewhat this value should be a more than 10 even we can explore also from the binding width calculations not required. I think here in the unknown concentration if you look at all the three samples which are the well concentration it calculated as a 30 point 38.7, 37.9 it is nothing but 38 microgram and 51.

It is somewhat 51 is something like a very high showing, but our standard curve is 50, but still the instrument extrapolates and calculates the concentrations. So, now, we can save this data, we can save this as a save report. If you save a report it will convert this into a excel sheet like you can give  apo standard curve then say.

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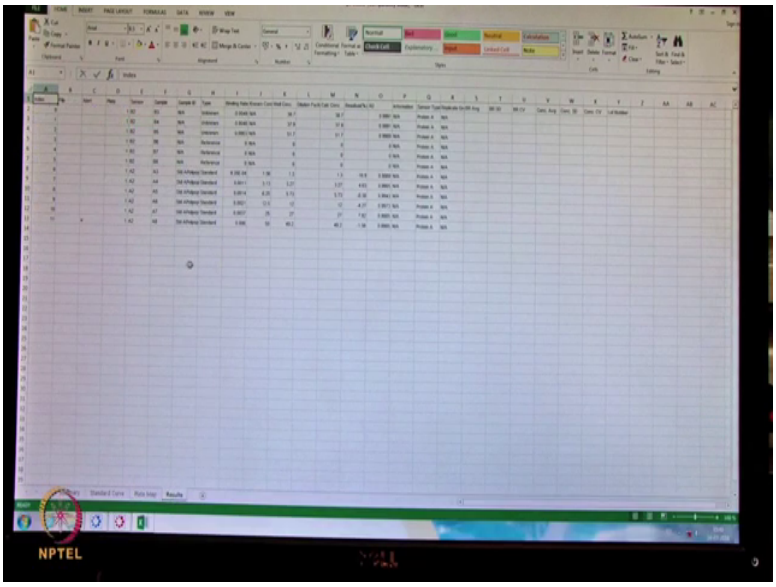

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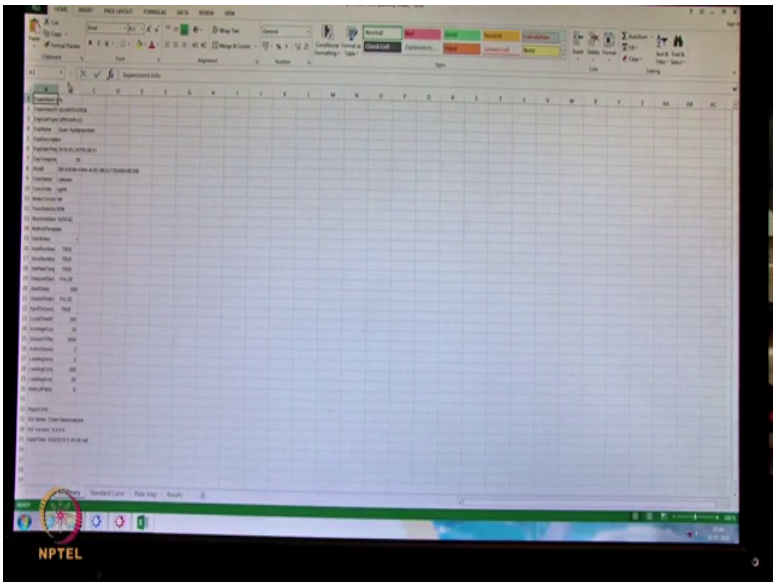
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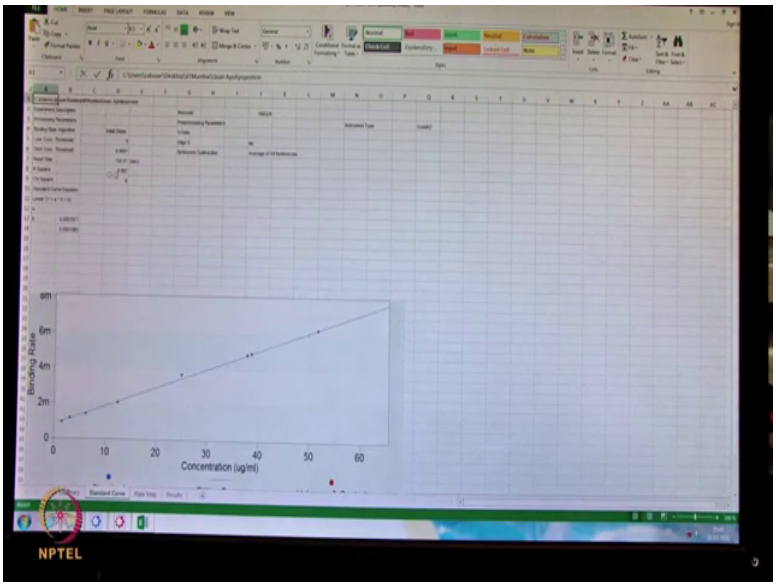


It will create as a excel sheet the report will be created as a excel sheet. In the summary you will see the what are all the data where it is and all that and the standard curve, if you look at this was the standard curve we had the standard curve also pi square is 0.997 r square and a chi square is a 0. This is a very good actually if you look at all data points almost wrong on the very near to a line.

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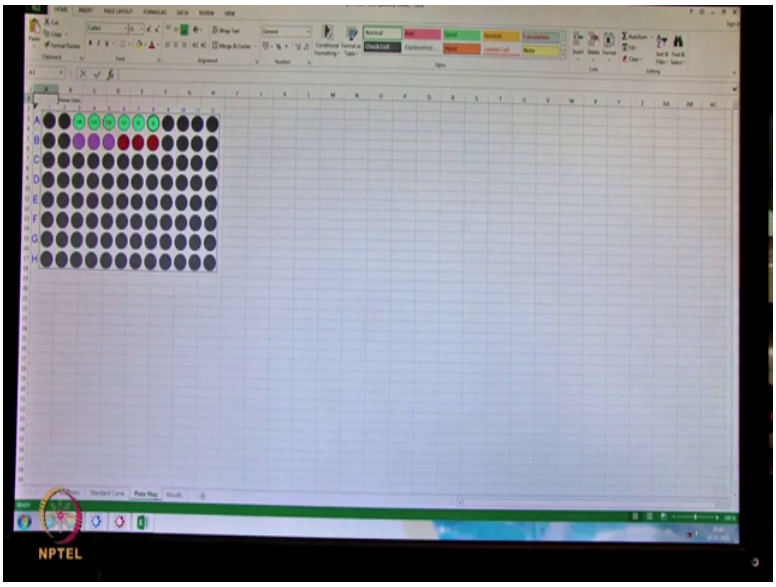


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And in the plate map where you had put your the samples and the unknown samples in the result section. This is your standard along with your percent residuals and also for the unknown samples here it is assigned unknowns that that indicated to your calculated concentration. If it is any like, if your sample is dilution diluted we had to put a dilution factor automatically calculate it is the calculated concentration with respect to a dilution factor. If you look at the Oscar values of the all the unknown samples as well as the standards it is a more than 0.9. So, the data is acceptable. So, with this I am going to finish the quantitation experiments thank you.

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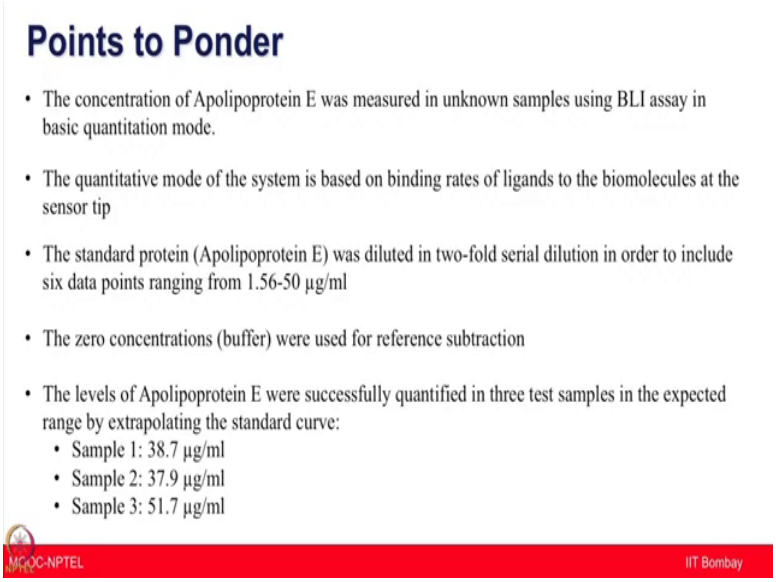


In today's lecture and demonstration session you have observed another application of bio layer interferometry. I hope you made an observation that is standard curve obtained for apolipoprotein E was linear and had good dynamic range with high response level. These standard curves can be extrapolated to find the concentration of apolipoprotein in the complex solution. Of course, we can choose any other protein of interest and the same experiment can be performed to measure the concentration of your protein of interest from the complex solution.

Bio layer interferometry could be used to measure the concentration of this specific protein even in complex solutions without needs to purify the proteins. In the next lecture we will see more of these applications of using label free biosensors to study biomolecular interactions.

Thank you.