ELISA Test Steps

ELISA is an acronym for enzyme-linked immunosorbent assay. It has been around since the 1970's and is now a regular part of many potato seed inspection programs where it is used for virus detection. The most crucial element of an ELISA is a highly specific antibody-antigen (PLRV or PVY) interaction. In a nutshell, ELISA for potato viruses tests for presence of a specific virus, such as PVY.

ELISA is used to detect a sample antigen, which is PVY. The most widely used ELISA format is the sandwich where the candidate sample is sandwiched between two antibodies. The sandwich ELISA is commonly used as it is sensitive and specific.

Typical steps in ELISA are:

The ELISA plate is prepared with a buffer.
A known quantity of capture antibody is bound onto it.
The plate is washed to remove unattached antibodies.
The sample of interest, such as PVY candidate leaf sap, is applied to the plate where the antibodies specific to PVY, capture it.
The plate is washed to remove unbound plant sap.
An enzyme conjugate (antibodies) is added to capture PVY in an "antibody sandwich."
The plate is washed to remove the unbound antibody-enzyme conjugates.
A chemical (developer) is added to be converted by the enzyme into a color.
The color (absorbance) of the plate wells is measured with a plate reader to determine the presence of PVY.

A positive and a negative control (blank with no plant tissue) is run with each ELISA plate. The negative control serves as a measure of background "noise." Specific washing protocols for the plates reduces and helps standardize the background noise. However, there is always some level of background interference.

To address this, a positive well a positive has to be more intense yellow than the background or negative well by a factor to be considered positive. The Entomology

lab at UMaine uses 2x, or twice the background color numeric reading of the negative well for a sample as a minimum to be considered positive for the virus in question.

It is also important to note that accurate recordkeeping is of paramount importance.

The following is the step-by-step procedure used by the UMaine Entomology lab for virus detection using a sandwich ELISA method.

Speaker 1 (00:05):

When collecting leaves, we need to be extremely accurate in labeling the bags. So for this bag, we have two 10, which represents the plot 27 represents the treatment number and 11 represents the 11th plant in this plot. So we will collect a leaf or a leaflet. We'll place it in the bag. We'll then transport it back to the lab refrigerator and get ready to be able to extract the second. So the leaf has collected and placed in this bag is from replication 200 plot, 10 it's treatment, 27. And it's the 11th plant in that. So from there, we will transfer that information onto the ELISA plate map, and we can see that's 210 27, 11 is in column 11.

Speaker 1 (01:05):

Yeah. So we can later find out if 210 to 27, 11 is positive or not based on the map that we've designed. So we now have brought the sample back to the lab. We're going to press it between the two rollers. If the leaf is dry, sometimes we need to add a few drops of distilled water to encourage the sap to be released from the leaf. We will then collect two or three drops of the sap could be later processed. And the final step is cleaning off the shafts of the grinder. So it does not cross contaminate. So the ELISA plate we're using today is provided to us from Agdia. And we're going to code it to test for potato leaf roll virus. We have to take 9 mils of distilled water, and I use a dropper bottle, which you'll see in a second. So I'll dispense 9 mils of distilled water and the dropper bottle.

Speaker 1 (02:22):

I will then get a smaller pipette tube. They will do 1 mill. Now I need to take the carbon coating buffer supplied by Agdia. I need a 1 mill of this. So, I dispense that 1 mil and to that 9 mils of distilled water for a total of 10 mills, getting rid of that, the next part we have to add is we have to add to capture anybody. So this is a much smaller measurement. We need 50 microliters. So we use a smaller pipette, smaller tips, and we will get 50 microliters dispense that in the tube.

Speaker 1 (03:36):

So from this point, I put my dropper tip back on. Some people will use a trough and they'll use a pipette to dispense all through out. I prefer the dropper, so I give it a quick little shake. So, I want to put two drops in each well and I start at the top and work my way across. I don't want to be near the sides. So I want the drop to be a true drop because that'll give me a hundred microliters. So I've coated every single well we'll get appropriate coating buffer. I then have two options. I can let this incubate in a moist chamber for four hours or let it incubate in the refrigerator overnight. So, I prefer to do it overnight. So, I'll put this in a Tupperware dish or any type of dish with a wet paper towel or a damp paper towel for humidity, with the cover put it into the refrigerator. And it'd be ready to go tomorrow morning. So I removed the ELISA plate that we started or coated yesterday from the refrigerator. And now we have to wash the plate three times. So I'm gonna take all the contents in here and dump it out in the sink with quick snaps. So we get rid of it. Now we're going to take this handy dandy water bottle, wash a plate three times.

Speaker 2 (06:02):

Shake it really good. I've got to get the bubbles and stuff out of the inside of that. So I got pat it and then pat it until the bubbles are all gone. Pretty good. So the next step is we have to coat this with plant material and buffer. So now that we've washed the plate, we now have to add a general extract buffer to the sample tissue that was previously ground up. And I use a repeater pipette, which will just dispense the correct proportion of buffer. So each well is treated the same for the entire plate. This repeater only allows where you get half the plate. So I got to come back and reload a second time. When I do this, I make sure that the tip never touches a well it'll a cross contaminate so that we've coded, or we put buffer in each one of the Wells it's ready for the next step.

Speaker 1 (07:48):

So now we bring the plate that we just washed to the part where we have to dispense the buffer and sap to the plate. Very important that we've made sure that A1 is in the top left corner, which coincides with A1, which is in the top left corner of the plates. Because if it's inverted, we're not going to have a correct reading and it'll still read, but we just won't be able to trace back that sample. So make sure everything is lined up. So for this, I'm using a multi-channel pipette and we're going to be dispensing 100 microliters of buffer plant solution into the well. So to do that easily, I get 12 pipette tips and insert them into the little vials and I pump it a couple of times here to get a good mixture. And then I just dispense it into the plate and so on and so forth until I get the whole plate done. It takes a little practice

to get 12 pipette tips lined up to the correct position that it needs to go in. Each time we get a new set of pipette tips to prevent contamination. So, this is what the plate looks like. Now I'll put the white contrast to that. So then I'll take this plate, I'll put it back in our humidity box, put a cover on it.

Speaker 1 (09:54):

We'll let it sit here on the counter side for two hours and we'll do the next step. I will keep this buffer and sap solution, as you can see, there's still solution in here in case something happens with this or it gets dropped or something happens. We can always come back to this one to do another plate, without having to grind the tissue all over again. So we have let the two hour time go by. This is the loaded plate that we loaded with a buffer and the plant tissue. Now we have to wash this out at a minimum four times, a maximum seven times. So just like we did before, we're going to shake out the contents.

Speaker 2 (10:49):

Shake it out, pat it until we get all the bubbles out.

Speaker 1 (10:57):

So now we're back to what looks like a clean plate. We'll put this back in the humidity box and we'll make our buffer. Okay. So next we have to make an, our enzyme conjugate. So to do that, this recipe calls for 8 mills of distilled water, and I'll use the same dropper bottle that I use. So I'm dispensing 8 mils of distilled water, And then I need for this test for, Potato leaf roll virus. I need to have 2 mils of an ECI buffer, which is provided by Agdia. And then the last step is I need the Alka phos enzyme conjugate, which is a much smaller measurement of 50 microliters.

Speaker 2 (12:17):

Cap the bottle and give it a shake.

Speaker 1 (12:21):

So now I have to coat the plate like we did in the previous steps, just a different solutions, same motion, two drops per well. So that plate is now filled. We'll put it back into the humidity box and it will incubate for two more hours. Okay? So for this step, we have to mix our PNP buffer to be eight mils distilled water

Speaker 1 (13:44):

And then we'll switch. And now we need to put two mils of PNP substrate buffer

Speaker 1 (14:05):

So after we get the liquid products in, we have to use PNP substrate tablets, and these tablets, the direction say don't touch them with the skin. So we'll use a little forceps, we'll take one, two. We will then cap this. We'll put this in a dark place. And then we will it let it set for about 10 minutes. The pills will have to all dissolve and then we will start to, coat the plate for the final time. So these little yellow tablets are what is going to be the dye that will signify if something has in this case. PLRV so the enzyme conjugate has been placed in the ELIZA plate, which had been placed in the humidity box. It's, been there for two hours. So now it's time to wash the plate again,

Speaker 1 (15:18):

And get it prepared for the PNP final step, shake it all out, wash the plate four times.

Speaker 2 (15:36):

So that's the fourth time we're going to get all the wash buffer out, pat it several times.

Speaker 1 (15:50):

We want to make sure there are absolutely no bubbles because this is where it'll cause a problem because the PNP buffer solution that we're going to put in here is going to potentially turn to a yellow color and then the plate reader will read it. So if there's a bubble in this, the plate reader will potentially have a misread

Speaker 1 (16:12):

So I've checked all them all. I'll put it back in the humidity box and we'll take it over to the desk to put the PNP buffer in.

Speaker 2 (16:22):

So the final step in getting the results of our ELISA test is putting the developer in. So it will glow yellow or light up as a yellow, if you will, that can be read by the plate reader. So she's putting the final PNP buffer in each well. We'll let this sit for one hour in the dark place and then we will read it with a plate reader.

Speaker 1 (16:56):

So I have my ELISA plates in a dark area. So I'm going take this ELISA plates over the plate reader. So if we look down inside that some are very, very faint. A human eye can see them. So we're going to actually put this in a more sophisticated

device called the plate reader. So I take the ELISA plate out of the humidity box. I'm going to wipe off the bottom, making sure there's no moisture. I'm going to place it in the ELISA plate reader on the ELISA plate reader, it's hooked to the computer. And I'm just going to tell it to start to scan

Speaker 2 (17:43):

The plate reader is going to take it in

Speaker 2 (17:53):

Going to read every single well.

Speaker 1 (18:14):

The objectives, and if we look at the computer screen, it gives us all kinds of information that we have to then analyze to see what's positive, what's negative. So all labs have different protocols. In this example, we have one, well that has illuminated yellow, and this is a positive control. The top left-hand corner for our lab is a negative control. So if we did not have this positive control here, all the other samples throughout this entire plate are all negative. So we would never know if the plate actually worked or not. So with this plate, it tells us the plate did his job. It found a positive and all the, our samples throughout this entire plate are all negative.