ELISA Plan

For collaboration with an immunology research laboratory

Ovalbumin (Chicken egg) Specific Antibody ELISA for testing serum samples

Day 1: Add OVA to the plate

Make ovalbumin solution that has a final concentration of 100ug/ml. It should be diluted in PBS (1X Phosphate Buffered Saline). The OVA concentrated stock solution should be at 10 mg/ ml.

Ex: Making 100ug/ml OVA for 5 plates from the 10 mg/ml OVA stock:
How much solution is needed to cover all the wells if we add 25ul/well?
(5 plates)(100 aprox wells on plate)= 500(25ul/well)=12500ul/1000=12.5 ml of solution

Put 12.5 ml of PBS into a tube. Now add the appropriate amount of 10 mg/ml OVA
For this example 125ul of 10mg/ml solution should be added to the 12.5 ml.

-The Tube should then be mixed well by inverting several times.
-Add OVA protein to the plate by adding 25ul of the OVA solution to each well using a pipette. Make sure the solution covers the bottom of the well. You may need to gently tap the sides of the plate in order for the liquid to move to the bottom of the well.
-Put the plate in a plastic Ziploc bag, roll up a paper towel wet it and put it in the bag with the plate. This will help stop evaporation. Close the bag eliminate most of the air inside.
-Carefully place the bag into a refrigerator overnight.

Day 2: Block the plate to fill in the empty space between the bound OVA

-Take a paper towel or equivalent and place it on the desk, fold it over once.
-Remove the plate from the bag; look to make sure there is still solution in each of the wells
-Take the plate to the sink and quickly turn it upside down to remove the OVA solution,
then pat the plate on the paper towel, upside down, until wells seems to be empty.
- Add 100ul to each well of the blocking buffer
buffer: 10% Fetal Calf Serum (FCS), in 1X PBS

The protein in the FCS will bind the unbound plastic not covered in OVA protein, thus creating an even layer across the bottom of the well. This will stop the antibodies in the in the sera, and the secondary antibodies, from binding to anything besides the OVA protein.

-Leave the plate at room temperature for 2 hours, or overnight in the refrigerator.
The plate should be in the Ziploc bag for this step with the wet paper towel.

Day 3: Add the serum

The serum was collected from mice that were immunized with OVA protein. This means they should have antibodies that are specific for OVA. There are three different tubes of serum:

1. From mice immunized once with OVA protein and blood collected 38 days later.
2. From mice immunized twice with OVA protein, on days 0 and 30. Blood was collected 8 days after the second immunization (day 38).
3. From mice that were not immunized.

-Take the plate out of the Ziploc bag. Keep the bag. Turn the plate upside down in the sink and shake off the blocking buffer.
-Wash the plate 3 times by pouring washing solution onto the plate, completely covering each well. Turn the plate upside down and shake off. Repeat three times. To wash: use a squeeze bottle containing washing solution or a basin containing the buffer.
Washing solution- 1x PBS with Tween
-After the third wash pat the plate on a paper towel until wash solution is gone.

-Important do not let the plate dry out completely, the next solution should be added immediately.
- Next add 148.5 ul to the top row (left to right) of the plate using 10%FCS in PBS, and then add 50ul in every well below the first row.
- This is done to create a dilution of the serum down the plate. You would expect the top row to be the most concentrated, then gradually less concentrated as you move down the plate. So the top well will contain more anti-OVA antibody from serum then the bottom well.
-Add serum to the top wells. Add 1.5ul of serum to each well, two wells for each sample. They are done in duplicate to confirm positive or negative results.
-Once the serum is in the top well containing the 150ul of 10%FCS in PBS you can then dilute the sample down the plate.
-Place a tip on the pipette and set it to 100ul. Put the tip into the first well and pipette up and down 5 times, try to not create bubbles. On the fifth time leave the tip full, containing 100ul. Move to the next row just below that well and do exactly the same mixing in the 100ul from the previous well. Pipette up and down 5 times then transfer 100ul to the next well. There will be 50ul remaining in each well. When you get to the last well, throw away the tip and the 100ul it holds. This is a serial dilution of 1.5 fold.
-Put the plate into the plastic Ziploc and re-wet the paper towel inside. The plate should sit in the refrigerator overnight.

Day 4: The Secondary Antibody and Detection

In order to visualize the amount of OVA that exists in each well a secondary antibody must be added. This antibody has alkaline phosphatase enzyme attached to one side and the other side attaches to the OVA antibody. The phosphatase substrate can then be added; if the secondary antibody is present it will turn yellow.
The antibody you will use recognizes all IgG subtypes.

-Make a 1-4000 dilution of the secondary antibody. You will need enough for 50ul per well. That's about 5.1 ml per plate.
Ex. Make secondary antibody for 5 plates at a concentration of 1-4000
(5)(5.1)= 25.5ml of 10%FBS PBS
Then add 6.35ul (1/4000) of the antibody, notice the 6.35 is in ul (1 ul is 1000 less then 1ml, or rather there are 1000ul in one ml) if we wanted a 1/1000 dilution we would add 25.5ul of antibody, but in fact we want 4 times less then that so 25.5/4= 6.35ul

-Take the plate out of the bag. Shake off the serum. Wash the plate 3 times with washing solution. Do this exactly the same as yesterday before the serum was added. Pat it dry on a paper towel.
-Add 50ul per well. Don't let the plate sit too long before adding the secondary antibody, you do not want the wells to dry out.
-Let the plate sit at room temperature for 2 hours inside the Ziploc bag.

Detection:
-In the last 30 minutes of waiting make up the detection substrate.
-This substrate is light sensitive so make it up quickly and store it in the dark until ready for use.
-.037grams of the enzyme is added to 22.5ml of glycine buffer
-Make enough to add 50ul per well.

-Once the incubation period is over take the plate back to this sink. This time wash it 5 times with washing solution. Fill the plate up with the washing solution, turn it upside down and shake off liquid. Repeat 5 times. It is important that all the unbound antibody is washed out of the wells or you may get a false positive. Pat the plate on a paper towel to dry.
-Invert the detection substrate to make sure it is mixed well
-Add 50ul per well of the substrate, wait about 5-10 min for it to develop
-If your serum samples contained anti-OVA antibodies the wells should turn yellow, the wells near the top should be brighter yellow because they received the most concentrated dose of serum.