

Laboratory Quick Guide

ELISA for Tracking Disease Outbreaks

Student Workstation Checklist

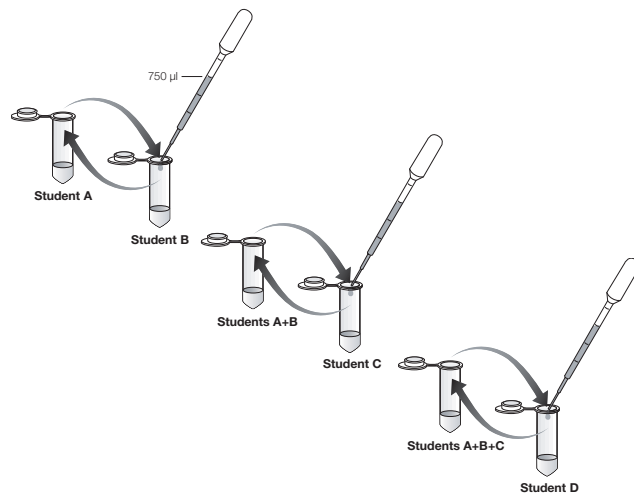
One workstation serves 4 students.

Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.75 ml)	4 (1 per student)	<input type="checkbox"/>
Violet tube (+)	Positive control (0.5 ml)	1	<input type="checkbox"/>
Blue tube (-)	Negative control (0.5 ml)	1	<input type="checkbox"/>
Green tube (PA)	Primary antibody (1.5 ml)	1	<input type="checkbox"/>
Orange tube (SA)	Secondary antibody (1.5 ml)	1	<input type="checkbox"/>
Brown tube (SUB)	Enzyme substrate (1.5 ml)	1	<input type="checkbox"/>
12-well microplate strips		2	<input type="checkbox"/>
50 μ l fixed-volume micropipet or 20–200 μ l adjustable micropipet		1	<input type="checkbox"/>
Yellow tips		10–20	<input type="checkbox"/>
Disposable plastic transfer pipets		5	<input type="checkbox"/>
70–80 ml wash buffer in beaker	Phosphate buffered saline with 0.05% Tween 20	1	<input type="checkbox"/>
Large stack of paper towels		2	<input type="checkbox"/>
Black marking pen		1	<input type="checkbox"/>

1. Label a yellow tube and a plastic transfer pipet with your initials.
2. Use the pipet to transfer all your “bodily fluid” sample into the tube of another student. Gently mix the samples, then take back half of the shared sample (750 μ l) to your own tube. Write down the name of the student next to “Sharing Partner #1”.
3. When instructed to do so, repeat the sharing protocol two more times. Discard this transfer pipet after this step.

Optional stopping point: Samples may be stored at 4°C overnight.

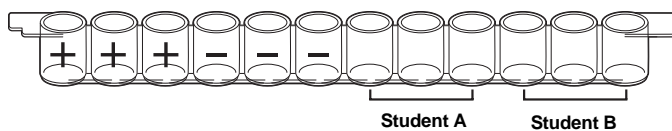
4. Label your 12-well strip. On each strip label the first 3 wells with a “+” for the positive controls and the next 3 wells with a “-” for the negative controls. Label the remaining wells with your and your lab partner’s initials (3 wells each).



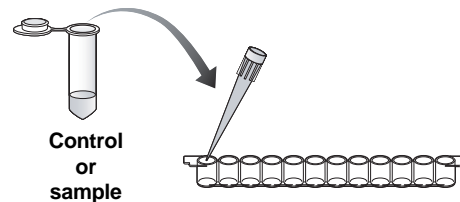
Sharing Partner #1 _____

Sharing Partner #2 _____

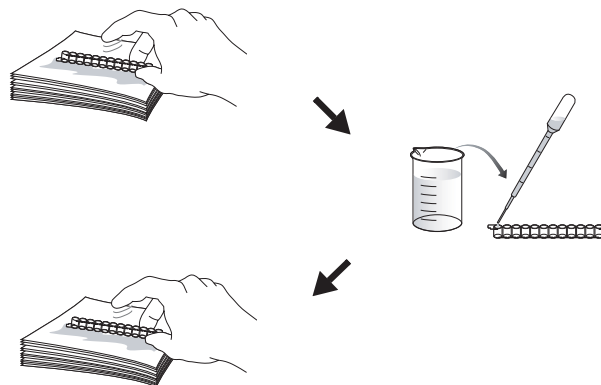
Sharing Partner #3 _____



5. Use a fresh pipet tip to transfer 50 μ l of the positive control (+) into the three "+" wells.
6. Use a fresh pipet tip to transfer 50 μ l of the negative control (-) into the three "-" wells.
7. Transfer 50 μ l of each of your team's samples from step 3 into the appropriately initialed three wells, using a fresh pipet tip for each sample.
8. Wait 5 minutes while all the proteins in the samples bind to the plastic wells.
9. WASH:



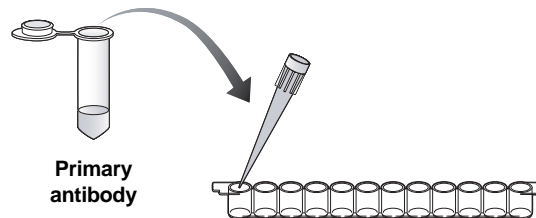
- a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid samples splashing back into wells.
- b. Discard the top paper towel.
- c. Use a fresh transfer pipet to fill each well with wash buffer, taking care not to spill over into wells. Note: the same transfer pipet is used for all washing steps.
- d. Tip the microplate strip upside down onto the paper towels and tap.
- e. Discard the top 2–3 paper towels.



10. Repeat wash step 9.

WASH

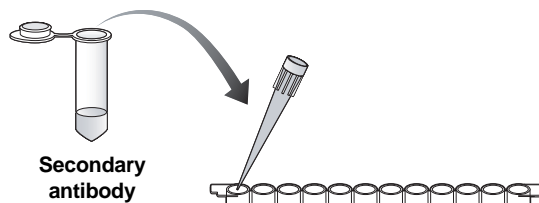
11. Use a fresh pipet tip to transfer 50 μ l of primary antibody (PA) into all 12 wells of the microplate strip.
12. Wait 5 minutes for the antibodies to bind to their targets.



13. Wash the unbound primary antibody out of the wells by repeating all of wash step 9 **two** times.

WASH 2x

14. Use a fresh pipet tip to transfer 50 μ l of secondary antibody (SA) into all 12 wells of the microplate strip.

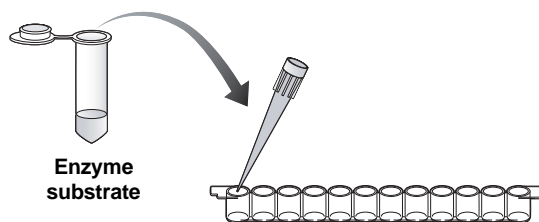


15. Wait 5 minutes for the antibodies to bind to their targets.

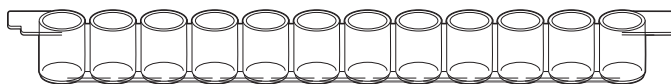
16. Wash the unbound secondary antibody out of the wells by repeating wash step 9 **three** times.

WASH 3x

17. Use a fresh pipet tip to transfer 50 μ l of enzyme substrate (SUB) into all 12 wells of the microplate strip.



18. Wait 5 minutes. Observe and record the results.





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