

Laboratory Quick Guide

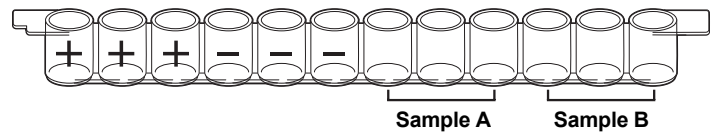
ELISA Antibody Test

Student Workstation Checklist

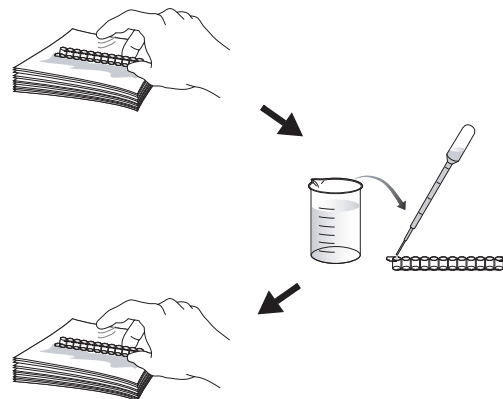
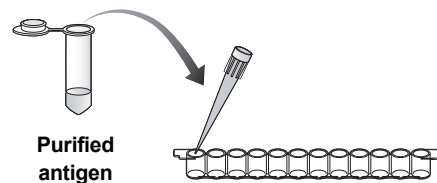
One workstation serves 4 students.

Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.25 ml)	4	<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 (AG)	Purified antigen (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 pipet		1	<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 the yellow tubes (if necessary) to identify the samples being tested.
2. 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 to identify the samples being tested (3 wells each).



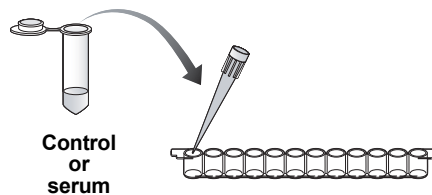
3. Use a fresh pipet tip to transfer 50 μ l of purified antigen (AG) into all 12 wells of the microplate strip.
4. Wait 5 minutes for the antigen to bind to the plastic wells.
5. WASH:
 - a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid splashing sample back into wells.
 - b. Discard the top paper towel.
 - c. Use your transfer pipet to fill each well with wash buffer, taking care not to spill over into neighboring 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.
6. Repeat wash step 5.
 7. Use a fresh pipet tip to transfer 50 μ l of the positive control (+) into the three “+” wells.

WASH

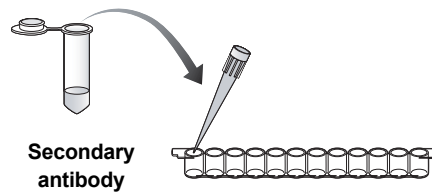
8. Use a fresh pipet tip to transfer 50 μ l of the negative control (–) into the three “–” wells.
9. Transfer 50 μ l of each of your team’s serum samples into the appropriately initialed three wells, using a fresh pipet tip for each serum sample.
10. Wait 5 minutes for the antibodies to bind to their targets.



11. Wash the unbound primary antibody out of the wells by repeating all of wash step 5 **two** times.

WASH 2x

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

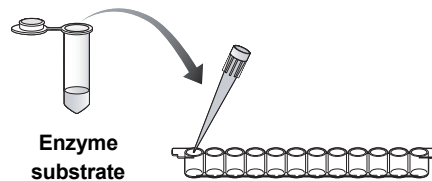


13. Wait 5 minutes for the antibodies to bind to their targets.

14. Wash the unbound secondary antibody out of the wells by repeating wash step 5 **three** times.

WASH 3x

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



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





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