

Laboratory Quick Guide

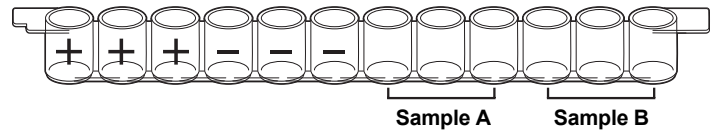
ELISA Antibody Test

Student Workstation Checklist

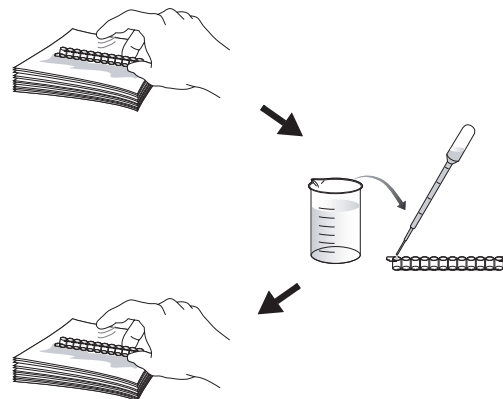
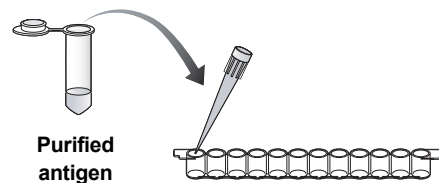
One workstation serves 4 students.

| 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).



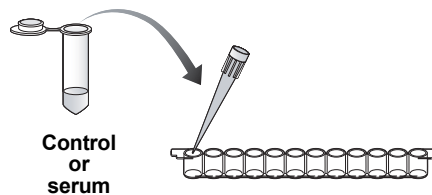
3. Use a fresh pipet tip to transfer 50 μ l of purified antigen (AG) into all 12 wells of the microplate strip.
4. Wait 5 minutes for the antigen to bind to the plastic wells.
5. WASH:
 - a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. 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

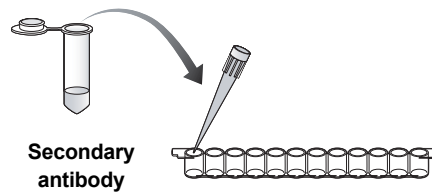
8. Use a fresh pipet tip to transfer 50 μ l of the negative control (–) into the three “–” wells.
9. Transfer 50 μ l of each of your team’s serum samples into the appropriately initialed three wells, using a fresh pipet tip for each serum sample.
10. Wait 5 minutes for the antibodies to bind to their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

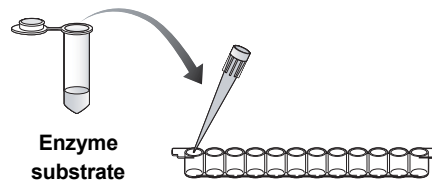


13. Wait 5 minutes for the antibodies to bind to their targets.

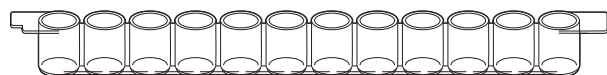
14. Wash the unbound secondary antibody out of the wells by repeating wash step 5 **three** times.

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.





**Bio-Rad
Laboratories, Inc.**

*Life Science
Group*

Web site www.bio-rad.com **USA** (800) 4BIORAD **Australia** 02 9914 2800 **Austria** (01)-877 89 01 **Belgium** 09-385 55 11 **Brazil** 55 21 2527 3454
Canada (905) 712-2771 **China** (86 21) 6426 0808 **Czech Republic** + 420 2 41 43 05 32 **Denmark** 44 52 10 00 **Finland** 09 804 22 00
France 01 47 95 69 65 **Germany** 089 318 84-0 **Greece** 30 210 777 4396 **Hong Kong** (852) 2789 3300 **Hungary** 36 1 455 8800
India (91-124)-2398112/3/4, 5018111, 6450092/93 **Israel** 03 951 4127 **Italy** 39 02 216091 **Japan** 03-5811-6270 **Korea** 82-2-3473-4460
Latin America 305-894-5950 **Mexico** 55-52-00-05-20 **The Netherlands** 0318-540666 **New Zealand** 64 9 415 2280 **Norway** 23 38 41 30
Poland + 48 22 331 99 99 **Portugal** 351-21-472-7700 **Russia** 7 095 721 1404 **Singapore** 65-64153188 **South Africa** 00 27 11 4428508
Spain 34 91 590 52 00 **Sweden** 08 555 12700 **Switzerland** 061 717 95 55 **Taiwan** (886 2) 2578 7189/2578 7241 **United Kingdom** 020 8328 2000
