Laboratory Quick Guide

Antigen Detection ELISA

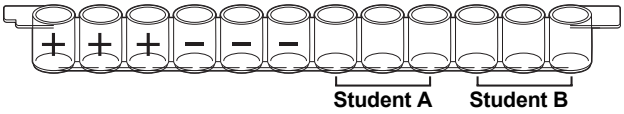
Student Workstation Checklist

One workstation serves 4 students.

Item (Label)	Contents	Number	(✔)
Yellow tubes	Student test samples (0.25 ml)	4	❑
Violet tube (+)	Positive control (0.5 ml)	1	❑
Blue tube (–)	Negative control (0.5 ml)	1	❑
Green tube (PA)	Primary antibody (1.5 ml)	1	❑
Orange tube (SA)	Secondary antibody (1.5 ml)	1	❑
Brown tube (SUB)	Enzyme substrate (1.5 ml)	1	❑
12-well microplate strips		2	❑
50 µl fixed-volume micropipet or 20–200 µl adjustable micropipet		1	❑
Yellow tips		10–20	❑
Disposable plastic transfer pipet		1	❑
70–80 ml wash buffer in beaker	Phosphate buffered saline with 0.05% Tween 20	1	❑
Large stack of paper towels		2	❑
Black marking pen		1	❑

1. Label the yellow tubes with each student's initials.

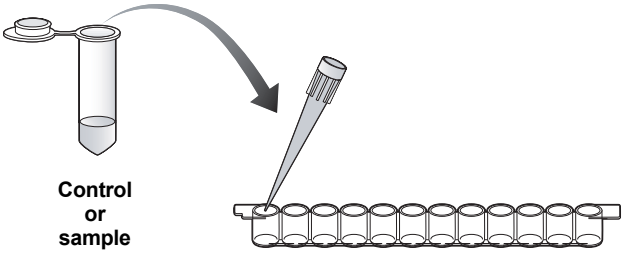
2. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "–" for the negative controls. Label the remaining wells with your and your partner's initials (3 wells each).



3. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.

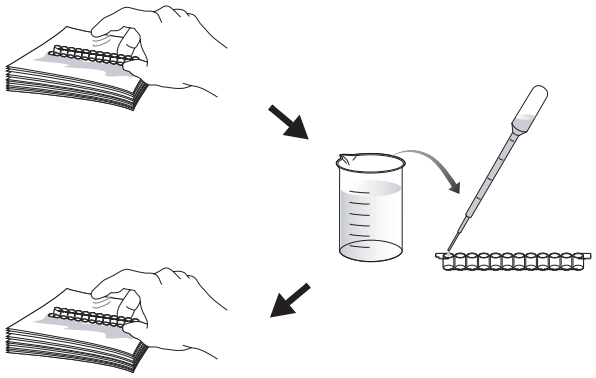
4. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.

5. Transfer 50 µl of each of your team's samples into the appropriately initialed three wells, using a fresh pipet tip for each sample.



6. Wait 5 minutes while the proteins in the samples bind to the plastic wells.

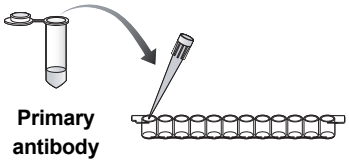
7. WASH:
    a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid splashing samples back into wells.
    b. Discard the top paper towel.
    c. Use your transfer pipet to fill each well with wash buffer, taking care not to 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.



8. Repeat wash step 7.

WASH

9. Use a <u>fresh</u> pipet tip to transfer 50 µl of primary antibody (PA) into all 12 wells of the microplate strip.

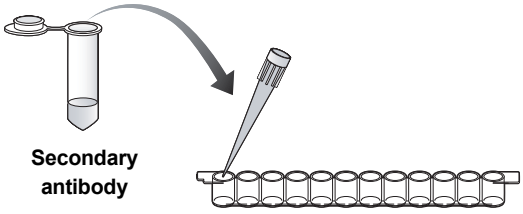


10. Wait 5 minutes for the antibodies to bind to their targets.

11. Wash the unbound primary antibody out of the wells by repeating all of wash step 6 **two** times.

WASH 2x

12. Use a <u>fresh</u> pipet tip to transfer 50 µl of secondary antibody (SA) into all 12 wells of the microplate strip.

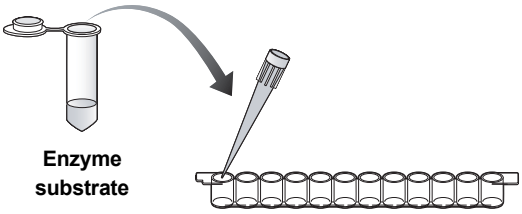


13. Wait 5 minutes for the antibodies to bind to their targets.

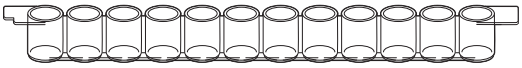
14. Wash the unbound secondary antibody out of the wells by repeating wash step 6 **three** times.

WASH 3x

15. Use a <u>fresh</u> pipet tip to transfer 50 µl 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*

Bulletin 3135 US/EG Rev B *05-0469 0505 Sig 1204*