

## ELISA PROTOCOL

1. Obtain a test tube containing “body fluids”. Record your tube number.
2. “Swap fluids” with 3 people. For each:
  - a. record the name (and tube number) of the person you’ll be swapping with.
  - b. add the fluid from your tube to your partner’s tube and mix it gently.
  - c. return half of the fluid to your tube.
  - d. repeat the process with two more students. Record each swap.

**If doing two-day ELISA, stop here.**

3. Use a pipet to transfer 50ul of your sample to one of the wells of your table’s microtiter plate.
4. Add one set of positive & negative controls to the plate.
5. Allow the plate to sit (incubate @ RT) for 5 minutes.
6. Empty the microtiter plate by inverting it on a paper towel and tapping the back of the plate gently.
7. Wash the wells: Fill with the PBST-Tween20 solution & empty as above. Repeat 2X.
8. Add 50ul of the primary antibody solution and let it sit for 5 minutes. Empty the wells.
9. Wash the wells: Fill with the PBST-Tween20 solution & empty as above. Repeat 2X.
10. Add 50ul of the secondary antibody-HRP solution and let it sit for 5 minutes. Empty.
11. Wash the wells: Fill with the PBST-Tween20 solution & empty as above. Repeat 2X.
12. Add 50ul of the substrate to the wells.
13. Watch for a color change to blue within 5 minutes of adding the substrate.