

General Microbiology

Biology 251

ELISA Assay Protocol

Introduction

You are about to perform an ELISA or enzyme-linked immunosorbent assay, a test that detects antibodies in your blood to determine if you have been exposed to a disease. When you are exposed to a disease agent, your body mounts an immune response. Molecules that cause your body to mount an immune response are called antigens, and may include components of infectious agents like bacteria, viruses, and fungi. Within days, millions of antibodies — proteins that recognize the antigen and bind very tightly to it — are circulating in your bloodstream. Like magic bullets, antibodies seek out and attach themselves to their target antigens, flagging the invaders for destruction by other cells of the immune system.

Where Is ELISA Used in the Real World?

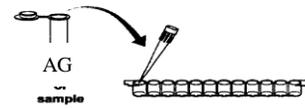
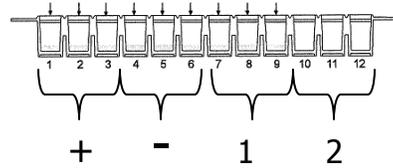
With its rapid test results, the ELISA has had a major impact on many aspects of medicine and agriculture. ELISA is used for such diverse purposes as pregnancy tests, disease detection in people, animals, and plants, detecting illegal drug use, testing indoor air quality, and determining if food is labeled accurately. For new and emerging diseases like severe acute respiratory syndrome (SARS), one of the highest priorities of the US Centers for Disease Control (CDC) and the World Health Organization (WHO) has been to develop an ELISA that can quickly and easily verify whether patients have been exposed to the virus. Over-the-counter kits that are based on the same principles as the ELISA include home pregnancy and ovulation tests, and tests for the presence of illegal drugs like marijuana.

Materials:

- Wash buffer
- A 12-well microplate strip
- Disposable sterile plastic transfer pipets
- 50ul volume micropipets and tips
- Stack of paper towels
- Marking pen
- Samples tubes
 - **Yellow tube** - the test sample (Numbered 1 - 12)
 - **Violet tube** – positive control labeled (+)
 - **Blue tube** – negative control labeled (-)
 - **Green tube** – Purified antigen labeled (AG)
 - **Orange tube** – secondary antibody labeled (SA)
 - **Brown tube** – enzyme substrate labeled (SUB)

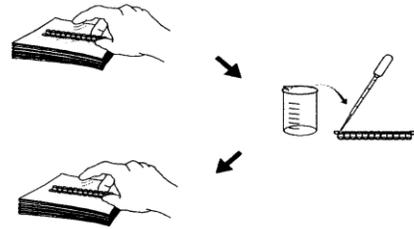
Procedure:

1. Take out your 12-well microplate
2. Label the side of the first 3 wells with (+) for your positive control, the next 3 wells with (-) for your negative control, and the next 3 wells with first sample number, the next 3 wells with second sample number, these are your test samples.
3. Get the **Green tube** (AG) with purified antigen and transfer 50ul of it into all 12 wells.
4. Wait 5 minutes for the antigen to bind to the wells.



5. The wash steps:

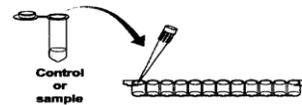
- Tip the microplate strip upside down onto the paper towels and **gently** tap the strip a few times to get rid of all the liquid. Be **careful** of samples splashing back into the wells.
- Throw away the top wet paper.
- Use a fresh transfer pipette and fill each well with Wash Buffer but don't spill over. The same pipette will be used for all washes.
- Tip microplate strip upside down on paper towel and tap just like before.
- Throw away top wet paper towels.
Repeat wash steps #5



WASH

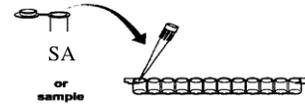
6. Transferring the samples steps:

- Get the **Violet tube (+)** and transfer 50ul of it into the positive control 3 wells.
 - Get the **Blue tube (-)** and transfer 50ul of it into the negative control 3 wells.
 - Get the Yellow tubes (samples) and transfer 50ul of each into the appropriate test sample wells.
7. Wait 5 minutes for the antibodies to bind to their targets.



8. Perform the Wash procedure in step #5 **two** times.
9. Get the **Orange tube** (SA) with the secondary antibody and transfer 50ul of it into all 12 wells
10. Wait 5 minutes for the antibodies to bind to their targets.
11. Perform the Wash procedure in step #5 **three** times.
12. Get the **Brown tube** (SUB) with the enzyme substrate and transfer 50ul of it into all 12 wells.
13. Wait 5 minutes.
14. Your positive control should have a blue color change and your negative control should have no change.
15. Observe and record your sample wells.

WASH 2X



WASH 3X

