

ELISA PROTOCOLS

Note: The researcher should optimize the precise conditions for a particular assay.

SOLUTION PREPARATION:Coating Solution:

Antigen or antibody is diluted in coating solution for immobilization onto the microplate. Commonly used coating solutions are: 50 mM sodium carbonate, pH 9.6; 20 mM Tris-HCL, pH 8.5; or 10 mM pBS, pH 7.2. A protein concentration of 1-10 ug/mL is usually sufficient.

Blocking Solution:

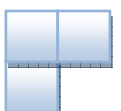
Commonly used blocking agents are: BSA, nonfat dry milk, casein and gelatin. Different assay systems may require different blocking agents.

Primary/Secondary Antibody Solution:

Primary/secondary antibody should be diluted in 1x Blocking solution to prevent non-specific binding. It is recommended to dilute antibodies between 1:100 and 1:500. Follow the manufacturer's advice for secondary antibodies.

Wash Solution:

Typically 0.1 M phosphate-buffered saline or Tris-buffered saline (pH 7.4) containing a detergent such as Tween 20 (0.02%-0.05% v/v).



Protocol:

1. Dilute the antigen to 1-2 ug/ml in coating solution
2. Add 100 ul of diluted antigen to appropriate wells. Incubate 2 hours at room temperature or 4 °C overnight.
3. Empty plate and tap out residual liquid.
4. Wash twice with 300 ul Wash solution.
5. Add 300 ul Blocking solution to each well. Incubate 1 hour.
6. Empty plate and tap out residual liquid.
7. Wash twice with 300 ul Wash solution.
8. Add 100 ul diluted primary antibody to each well. Incubate 1 hour at 37 °C or 3 hours at room temperature.
9. Empty plate, tap out residual liquid.
10. Fill each well with Wash solution. Invert plate to empty, tap out residual liquid. Repeat 3 times.
11. Add 100 ul diluted secondary antibody to each well. Incubate 1 hour at room temperature.
12. Empty plate, tap out residual liquid and wash as described in step 10.
13. Give final 5 minutes soak with Wash solution. Tap residual liquid from plate. This washing step is critical to reduce signal background.
14. Fill each well with Wash solution. Invert plate to empty, tap out residual liquid. Repeat 5 times.
15. Dispense 100 ul of substrate (e.g. pNPP) into each well. Develop the color for 30 minutes and immediately read plate with plate reader at 405-410 nm.

