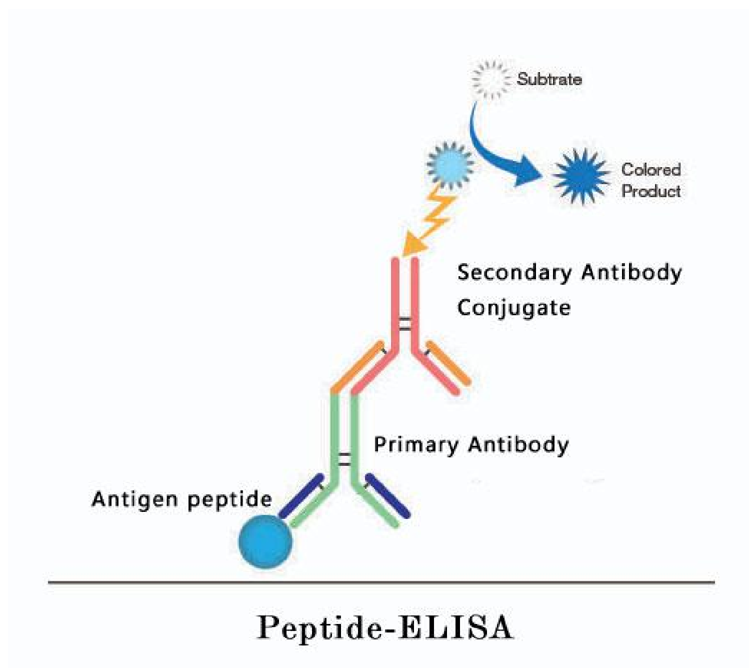
Peptide-ELISA Protocol

ELISA (enzyme-linked immunoSorbent assay) is an immunoassay that detects antigens via antibody binding that are linked to enzymes to allow for a diverse choice of detection methods. The peptide-ELISA detects antigen peptides that have been immobilized onto solid surfaces, commonly in the form of microplates. Once non-specific sites are blocked, primary antibodies and then enzyme conjugated secondary antibodies are incubated to build a complex for detection. Detection methods can include colorimetric, fluorometric, or luminometric depending on the type of secondary antibody conjugation.



Materials:

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 Buffer 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, TMB) into each well. Develop the color for 30 minutes.

16. Stop reaction if necessary and read plate with plate reader at the correct wavelength.