Chicken immunization with Keyhole limpet hemocyanin (KLH)-gp120 fragment (254-274) conjugate raises anti-KLH antibodies in egg yolks.

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ABSTRACT

Chicken immunization with peptides is ineffective if only just the peptides are being inoculated. However, to make the immune response effective the fragment 254-274 of HIV-1 was conjugated with a carrier protein (KLH) that produced a critical immune response, assessed by ELISA, Immunoblot analysis and dot blot [1-4]. The Polson method (1990) can be used effectively to separate the IgY antibody from the egg yolk of immunized chickens [5].

References


Two healthy layer chickens (brown Leghorn), aged approximately 6 months, are injected intramuscularly at multiple sites on the breast with the peptide-keyhole limpet hemocyanin (KLH) conjugate.

The chickens are immunized on day 0, with 0.2 µmol/ml of the fragment 254-274 of HIV gp120-conjugated to KLH (immunogen) in 0.5 ml complete Freund’s adjuvant (Sigma-Aldrich Co, St. Louis Missouri).

On days 15, 60, and 90 chickens are immunized with 0.2 µmol/ml of the immunogen in 0.5 ml incomplete Freund’s adjuvant (Sigma-Aldrich Co, St. Louis Missouri).

The eggs are collected post-immunization. The immunoglobulin Y is separated using the Polson method (1990).

The 96 well microtitre plate is coated overnight at 4°C with 1 µg/µl per well of a mixture of Keyhole limpet hemocyanin (KLH) in carbonate-bicarbonate buffer pH 9.6.

Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.

50 µl of water soluble fraction from eggs is added and incubated for 1h at room temperature and the microplate is rewashed 4X with PBS-Tween.
8. Then 50 µl of rabbit anti-chicken IgY-peroxidase conjugate diluted 1:30,000 in PBS-non-fat milk is added to each well and incubated for 1h at RT. The plate is washed 4X with PBS-Tween.

9. 50 µl of 4 mg/ml o-phenylenediamine solution (OPD) is added and the plate is incubated 15 minutes at RT in the dark.

10. The reaction is stopped with 50 µl of 3M H2SO4 solution.

11. The plate is visually assessed for the development of colour and read in a microplate reader at 492 nm.

12. A cut-off point should be calculated as the mean of the optical density of negative controls x 2 SD. The higher the OD value the higher will be the quantities of anti-KLH in hens.