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

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Abstract

Chicken immunization with peptides is inefective 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

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3. Collins AM, Roberton DM, Hosking CS, Flannery GR (1991) Oral immunization with xenogeneic antibodies stimulates the production of systemic and mucosal anti-idiotypic antibodies. Immunology 73: 388-393.

4. Ho DD, Kaplan JC, Rackauskas IE, Gurney ME (1988) Second conserved domain of gp120 is important for HIV infectivity and antibody neutralization. Science 239: 1021-1023.

5. Polson A (1990) Isolation of IgY from the yolks of eggs by a chloroform polyethylene glycol procedure. Immunol Invest 19: 253-258.

Materials

MATERIALS

- 5 × 2ml No-Waste™ Freund's Incomplete Adjuvant (FIA) **G-Biosciences Catalog #**786-099
- 2ml No-Waste™ Freund's Complete Adjuvant (FCA) **G-Biosciences Catalog #**786-709
- Fragment 254-274 of Gp120 of HIV (peptide)

1 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.

2 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).

3 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).

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

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

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

7 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 H_2SO_4 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.