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

Angel A Justiz-Vaillant<sup>1</sup><sup>1</sup>University of the West Indies St. Augustine**1** Works for me [dx.doi.org/10.17504/protocols.io.bjvtn6n](https://dx.doi.org/10.17504/protocols.io.bjvtn6n)[University of the West Indies](#) [angel.vaillant@sta.uwi.edu](mailto:angel.vaillant@sta.uwi.edu)Angel Justiz-Vaillant  
University of the West Indies St. Augustine

## 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

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## DOI

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MATERIALS

NAME	CATALOG #	VENDOR
5 x 2ml No-Waste™ Freund's Incomplete Adjuvant (FIA)	786-099	G-Biosciences
2ml No-Waste™ Freund's Complete Adjuvant (FCA)	786-709	G-Biosciences
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 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.