AUG 17, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bjvtkn6n

Protocol Citation: Angel A Justiz-Vaillant 2020. Chicken immunization with Keyhole limpet hemocynin (KLH)-gp120 fragment (254-274) conjugate raises anti-KLH antibodies in egg yolks.. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bjvtkn6n>

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Protocol status: Working
 We use this protocol and it's working

Created: Aug 17, 2020

Last Modified: Aug 17, 2020

PROTOCOL integer ID:
 40595

🌐 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 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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MATERIALS

MATERIALS

5 x 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) **Contributed by users**

- 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.