Aug 12, 2020

Chicken immunization with KLH-gp120 fragment (254-274) conjugate.

DOI
dx.doi.org/10.17504/protocols.io.bjnjkmcn

Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

University of the West In... angel.vaillant@sta.uwi.e...

Angel A Justiz-Vaillant
University of the West Indies St. Augustine

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

Protocol Citation: Angel A Justiz-Vaillant 2020. Chicken immunization with KLH-gp120 fragment (254-274) conjugate.. **protocols.io https://dx.doi.org/10.17504/protocols.io.bjnjkmcn**

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

Created: August 12, 2020

Last Modified: August 12, 2020

Protocol Integer ID: 40363

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

1. Schade R, Pfister C, Italatsch R, Henklien P (1991) Polyclonal antibodies from chicken egg yolk-an alternative to the production of mammalian IgG type antibodies in rabbits. ATLA 19: 403-419.

2. Justiz Vaillant AA, Akpaka PE, Smikle M, McFarlane-Anderson N (2012) In vitro Inhibition of Staphylococcus aureus Isolates by Anti-Anti-Idiotypic Antibodies to Staphylococcal Protein (SpA). J Vaccines Vaccin 3: 127-131.

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).