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Purification of avian egg yolk immunoglobulins using the water dilution method, precipitation with isopropanol and using HiTrap™ Columns.

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1 Works for me dx.doi.org/10.17504/protocols.io.bkbnksme

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1 Separate egg yolk from the egg white.

2 Add nine parts of distilled water to one part egg yolk.

3 Mix and stir slowly for 6.30 h at 4°C.

4 Centrifuge at 10 000 × g, at 4°C for 25 min to precipitate the lipids.

5 Collect the supernatant containing the IgY (water soluble fraction).

6 Slowly add 5 ml of cold isopropanol to the preparation, stirring constantly, and centrifuge at low spead.

7 Dilute the precipitate in PBS and adjust pH to 7.6. Calculate the protein concentration by ELISA. spectrophotometry or Bradford method.

8 Determine the purity of the preparation by SDS-PAGE.

9 Fill the syringe or pump tubing with de-ionized water. Remove the stopper and connect HiTrap™ column to syringe (use the connector supplied).

10 Snap off tab on the column outlet.

11 Wash out the ethanol with 26 ml of de-ionized water.

12 Equilibrate column with 26 ml of binding buffer. The recommended flow rate is 5ml/min.

13 Apply the IgY sample using a syringe fitted to Luer connector or by pumping it onto the column.

14 For better results, use a flow rate of 0.5 to 5.1 ml/min during sample application.

15 Wash with at least 51 ml of binding buffer or no material remains in the effluent.

16 Maintain a flow rate of 5 to 11 ml/min for washing.

17 Elute with 51 ml of elution buffer using a one-step or using a linear gradient though larger volumes are often required to break the interaction.

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18 After elution, regenerate the column by washing with 36 ml of wash buffer.

19 Re-equilibrate the column with 26 ml of binding buffer.

20 The column is now prepared for a new purification.

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