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An easy chromatographic method for purification of Immunoglobulin Y (IgY) using HiTrap™ Columns.

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

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

2 Snap off tab on the column outlet.

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

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

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

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

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

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

9 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.

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

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

12 The column is now prepared for a new purification.