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Purification of the any avian IgY using chloroform, trichloroacetic acid and affinity purification using HiTrap™ Columns.

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

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ABSTRACT

This IgY purification technology can be used to purified IgY from the egg yolk of any bird as ducks, bantam hens, ostrich, chicken, pigeon, pheasant, quail, guinea hen, cattle egret and goose.

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MATERIALS

NAME	CATALOG #	VENDOR
500ml Trichloroacetic acid (TCA) Solution, 10% (w/v)	786-886	G-Biosciences
Chloroform	319988	Sigma
PBS		Life Technologies
Affinity Purification Using HiTrap™ Columns		

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1 The IgY fraction of bantam hen was isolated by the chloroform-Trichloroacetic acid (TCA) -affinity chromatography method.

2 The egg is washed with warm water and the egg yolk is separated from the egg white.

3 The membrane is broken and the egg yolk collected.

4 The egg yolk is then diluted 1:3 in phosphate buffered saline (PBS), pH 7.4.

5 To 1/3 of the egg yolk mixture an equal volume of chloroform is added,

6 The mixture is then shaken and centrifuged for 30 min (1000×g, RT).

7 The supernatant is decanted and drop 1 ml of cold TCA and let the sample stand for 30 min at RT.

8 The mixture is then centrifuged as previously described.

9 The precipitate containing IgY is dissolved in PBS (pH 7.4) at a volume equivalent to 1/6 of the original volume of the egg yolk.

10 After that the preparation is dialyzed against 1L of PBS (pH: 7.4 for 24 h at 4°C).

11 The IgY is then removed from the dialysis tubing.

12 IgY concentration is assessed by an available methodology.

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13 IgY samples then are further purified by affinity chromatography.

14 The preparation can be stored at –20°C.

15 By a sandwich ELISA the IgY concentration can be assessed.

16 Use Affinity Purification Using HiTrap™ Columns as follows:

17 Fill the syringe with distilled water.

18 Connect the column to the syringe

19 Snap off the tab on the column outlet.

20 Wash out the ethanol with distilled water.

21 Equilibrate the column with binding buffer.

22 The recommended flow rate is 5 ml/min*.

23 Apply the sample using a syringe linked or fitted to Luer connector

24 Use a flow rate of 1 to 5 ml/min during sample application

25 Wash with at least 51 ml of binding buffer.

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26 Maintain a flow rate of 10 ml/min for washing.

27 Elute with 50 ml of elution buffer.

28 After elution, regenerate the column by using wash buffer

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

30 Store it until a new purification

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