

Aug 13, 2020

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

DOI

<https://dx.doi.org/10.17504/protocols.io.bjnskme>

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Protocol Citation: Angel Justiz-Vaillant 2020. Purification of the any avian IgY using chloroform, trichloroacetic acid and affinity purification using HiTrap™ Columns.. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bjnskme>

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Protocol status: Working

We use this protocol and it's working

Created: August 13, 2020

Last Modified: August 13, 2020

Protocol Integer ID: 40370

Keywords: purification of the any avian igy, igy purification technology, igy from the egg yolk, purified igy, avian igy, purification, affinity purification, igy, guinea hen, bantam hen, egg yolk, bird as duck, bird, using chloroform, chicken, trichloroacetic acid

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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.

Materials

MATERIALS

⊗ 500ml Trichloroacetic acid (TCA) Solution, 10% (w/v) **G-Biosciences Catalog #786-886**

⊗ Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988**

⊗ PBS **Life Technologies**

⊗ Affinity Purification Using HiTrap™ Columns



- 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.
- 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.
- 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