

Aug 25, 2020

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

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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 Sml/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.	5	Collect the supernatant containing the IgY (water soluble fraction).
Bradford method. Bradford met	6	Slowly add 5 ml of cold isopropanol to the preparation, stirring constantly, and centrifuge at low spead.
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 Sml/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.	7	
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7 Elute with 51 ml of elution buffer using a one-step or using a linear gradient though larger volumes are often required to	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	

18	After elution,	regenerate the o	column by wasl	hing with 36 r	nl of wash buffer.
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- 19 Re-equilibrate the column with 26 ml of binding buffer.
- $20 \quad \text{ The column is now prepared for a new purification.} \\$