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12 Ion Exchange

TJUSLS China[1]

[1]Tianjin University

1 Works for me dx.doi.org/10.17504/protocols.io.5e5g3g6

TJUSLS China
Tianjin University

GUIDELINES

Refer to the operating instructions of AKTApure.

MATERIALS

NAME	CATALOG #	VENDOR
AKTApure	/	Ge Healthcare
20% ethanol	/	
Buffer A(50mM Tris HCl pH=8.0)	/	
Buffer B(50mM Tris HCl1MNaClpH=8.0)	/	

BEFORE STARTING

1. Keep the protein sample on ice all the time in order not to inactivate it.
2. Use the reset programs stored in computer instead of setting it each time.
3.Check the front pressure of the column to prevent damage to the machine.

1 According to the predicted pI of the protein and the pH of the ion-exchange column buffer, firstly select the appropriate ion exchange column (anion exchange column or cation exchange column). The pH of buffer should deviate from the isoelectric point of the protein.

2 The protein is concentrated with a 10KD concentration tube, and then the exchange buffer is used to exchange the protein to the ion-exchange liquid A. Finally, it is concentrated to less than 5ml by centrifuging at 4°C and 3400rpm for 10 minutes in a high-speed centrifuge to remove insoluble substances and bubbles.

5 ml 4 °C 3400 rpm 00:10:00

3 Balance the selected column with liquid A.

4 Through the AKTApure protein purification system, the samples are loaded to the column at a flow rate of 0.5ml/min, and continue washing for 5min.

00:05:00

5 Gradually increase the content of liquid B in the column, change the salt concentration and then change the interaction between the sample and the column, and collect the corresponding eluent according to the position of the peak.

6 Use SDS-PAGE to check the result.

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