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13 Gel filtration

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Works for me

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GUIDELINES

Refer to the operating instructions of AKTApure.

MATERIALS

NAME	CATALOG #	VENDOR
AKTApure	/	Ge Healthcare
buffer(25mMTris 150mMNaCl pH8.0)	/	

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.

Choice of Gel

- 1 According to the size of the target protein, firstly select the appropriate gel to adapt to the size of protein.

Column equilibrium

- 2 Connect the proper column which adapts to target protein to AKTA high pressure tomographic system, use buffer of gel filtration chromatography (buffer: 25mMTris 150mMNaCl pH8.0) to prebalance the column of gel filtration chromatography of about 1.5 columns.

Sample concentration

- 3 Concentrate the protein to less than 1mL by centrifuging at 4°C, 3400rpm for 10 minutes in a high-speed centrifuge to remove insoluble substances and bubbles.

4 °C 00:10:00 1 ml

Add Sample

- 4 Add the protein to the column of gel filtration chromatography through a 1mL sample collecting loop. The protein is eluted at a flow rate of 0.5mL/min. The eluent was collected according to the peak position.

1 ml

- 5 Use SDS-PAGE to check the result.



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