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11 Concentration and fluid exchanging

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1 Works for me dx.doi.org/10.17504/protocols.io.5e6g3he

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GUIDELINES

1.Protein is an active molecule, ensuring it is always on the ice and clinging to the principle of freezing and melting quickly.
2.When the protein is concentrated, it should be inverted several times after each protein added and mixed.
3.When pipetting the protein with a pipette, tilt the concentrating tube at the angle of 45 degrees. The tip of the pipette tip is drawn against the corner of the upper chamber of the concentrating tube. Try not to touch the filter.

MATERIALS

NAME	CATALOG #	VENDOR
DTT	SV-DTT	P212121
EDTA		
NaOH		
20% ethanol	/	
Concentrating tube	/	

BEFORE STARTING

1.The concentrating tubes has different pore sizes, and it is preferable to select a pore size smaller than one third of the molecular weight of the interest protein.
2.Pre-cooled to 4 °C before use the thermo centrifuge.

4 °C

Concentrate

1 1. The default concentrator is already inactive.
2. Pre-cool the Thermo centrifuge to 4 °C in advance.

4 °C

3. Pour the protein solution to be concentrated into a concentrating tube, level it with the trim tube, place it in a Thermo centrifuge at 3400 rpm, stop at half an hour, pour off the effluent, and add the protein solution to the upper layer.

4. If the protein is used for long crystals, it can be concentrated to less than 500 µl. According to the amount of protein and protein performance, it is determined that the protein is colloid rather than turbid liquid. If the protein is used for 24 ml molecular sieves, concentrate to within 1 ml.
5. Pipet the concentrated protein into a Ep tube with a 200 µl pipette (with a yellow tip).

200 µl

Disposal of the reuse of the concentrating tube

2 1. Rinse the upper chamber of the used concentrator tube with distilled water several times, and add some hypersaline solution overnight at room temperature.
2. Pour off the sodium hydroxide from the upper chamber, rinse several times with distilled water, and centrifuge 3000 rpm for 5 minutes with distilled water.

00:05:00

3. Step 2 is repeated 3 times.
4. Add 20% ethanol or ddH_2O to the upper chamber and store at 4 °C.

4 °C

change the liquid

3 The steps are the same as the concentration, and the buffer is added without any what.