Protein Production & Assays

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Protocol

Protein purification from large sample volumes with WET FRED

Flow **RE**gulation **D**evice

1 INTRODUCTION

In mammalian or insect cell expressed recombinant proteins are often secreted in the cell culture medium thus the protein is present in a large volume before the purification step. To efficiently purify Strep-tag®II or Twin-Strep-tag® proteins the use of gravity flow columns is recommended over batch. That means, a large volume has to be applied to a gravity flow column which, depending on its size, has a certain flow rate. To facilitate this step, we have developed the WET FRED device allowing the transfer of large volumes, e.g., cell culture supernatants, to a Strep-Tactin® or Strep-Tactin®XT gravity flow column for purification of the recombinant target proteins. It works simply by hydrostatic pressure (siphon principle). Its small size and flexibility make it usable at the bench, in the cold room or in the fridge. The complete sample is applied due to the last drop filter, and during the application, the column does not need supervision since the column cannot run dry as long as the WET FRED is connected. Furthermore, no sophisticated software is necessary facilitating set up and use.

2 COMPATIBILITY AND COMPONENTS OF THE WET FRED SET

The WET FRED is compatible with all Strep-Tactin® and Strep-Tactin®XT gravity flow columns. The WET FRED Set is available in two formats, one for 1 ml gravity flow columns (Cat. No. 2-0911-001) and the other for 5-10 ml gravity flow columns (2-0910-001). The Wet Fred Set consists of the following items:

	Column adapter for 1 ml or 5-10 ml gravity flow columns		6 cm tube		20 ml syringe
	2x female luer adapter		100 cm tube		5 ml syringe
	2x male luer adapter		Bottle neck adapter		Last drop filter

3 COLUMN AND SAMPLE PREPARATION

Equilibrate a Strep-Tactin® or Strep-Tactin®XT gravity flow column as or described in the related protocol for Strep-Tactin® purification or Strep-Tactin®XT purification. After equilibration pause the protein purification protocol for Strep-Tactin® or Strep-Tactin®XT and assemble the WET FRED (4.). The protocols are available at https://www.iba-lifesciences.com /resources/download-area/.

If the sample contains free biotin and should be applied to a Strep-Tactin® gravity flow column, please note that prior to application free biotin has to be masked by BioLock containing avidin. Avidin binds free biotin and mask it, whereby the Strep-tag®II and Twin-Strep-tag® of a target protein is not bound. Otherwise, free biotin will bind to the engineered biotin binding pocket of Strep-Tactin® and reduce the binding capacity of the resin for Strep-tag®II or Twin-Strep-tag® target proteins. However, the binding capacity of Strep-Tactin®XT resins is not influenced by free biotin. A protocol for biotin blocking with a list of media containing biotin is provided at https://www.iba-lifesciences.com/resources/download-area/.

4 WET FRED ASSEMBLY

A video of the WET FRED assembly is available at
https://www.youtube.com/watch?v=6ZUbkfy_mNs

4.1 Close the column outlet with the cap.

4.2 Plug column adapter to the top of the column. Wet seal with water prior to plugging to facilitate insertion.

4.3 Gently plug 5 ml syringe via a female luer adapter to the 6 cm tube and plug the other end of the tube via a male luer adapter to the column adapter.

4.4 Insert one end of the 100 cm tube into the last drop filter. Wet one end of the tube with water to facilitate insertion. Gently plug the other end of the 100 cm tube to the column adapter via a male luer adapter.

4.5 Place the last drop filter on the bottom of the container filled with the sample.

4.6 Place the column via the bottle neck adapter into an appropriate bottle.

4.7 Gently aspirate the sample with the 5 ml syringe until the column bed is overlaid with 0.5-1 ml of sample (see arrow, Fig. 1, A).

4.8 Remove end cap from column outlet and collect flow through with an appropriate bottle.

4.9 Regulate flow to approximately 1 ml/min by adapting height difference between column outlet and sample surface (see Fig 1, B). 20 drops equal approximately to 1 ml. 1 ml/min fits for all columns. In case of larger volumes, 5 ml and 10 ml columns can be run up to 3 ml/min. 15 cm height difference might be a good starting point, but please note that depending on the form of the selected container (small and high or wide and flat) the liquid level might change faster or slower by time and therefore the flow rate.

4.10 Check after 20 min whether the sample volume over the column bed (0.5-1 ml) remains stable.

In case of volume increase, the system is not tight. Connections of luer adapters and correct fit of the sealing ring have to be checked in this case. Last drop filter ensures sample application to completeness and avoids clogging as well as dry running of the column.

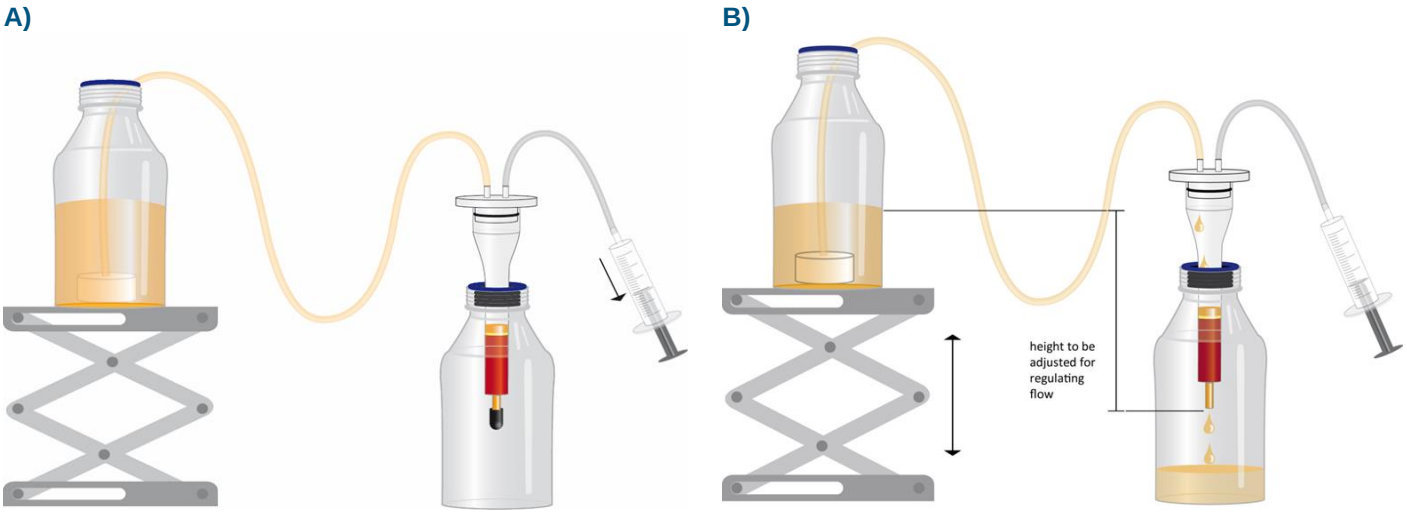

Fig 1: Assembly of the WET FRED and regulation of the flow speed.

5 DISCONNECT THE WET FRED

After application of the whole sample WET FRED can be disconnected or further used in case of 5-10 ml gravity flow columns. In the case of further use, replace the bottle containing the flow-through by a new one to collect the wash fraction separately.

5.1 Disconnect tubes from column adapter.

5.2 Remove column adapter from column and proceed with the protocol for Strep-Tactin® or Strep-Tactin®XT purification.

6 CLEANING OF THE WET FRED

6.1 Plug the 100 cm tube to the female luer adapter on the 20 ml syringe.

6.2 Transfer last drop filter into a beaker filled with water and aspirate at least 1x 20ml with the 20 ml syringe and eject.

6.3 Transfer last drop filter into a beaker filled with 30 ml ethanol and aspirate at least 1x 20 ml with the 20 ml syringe and eject.

6.4 Aspirate air to remove ethanol from last drop filter and tube.

6.5 Clean column adapter by rinsing with water and ethanol.

6.6 Disassemble the parts and let dry prior to the next purification run.

Check our Downloads page

www.iba-lifesciences.com/download-area.html

for the latest version of this manual.

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www.iba-lifesciences.com/patents-licenses-trademarks.html

If you have any questions, please contact

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We are here to help!