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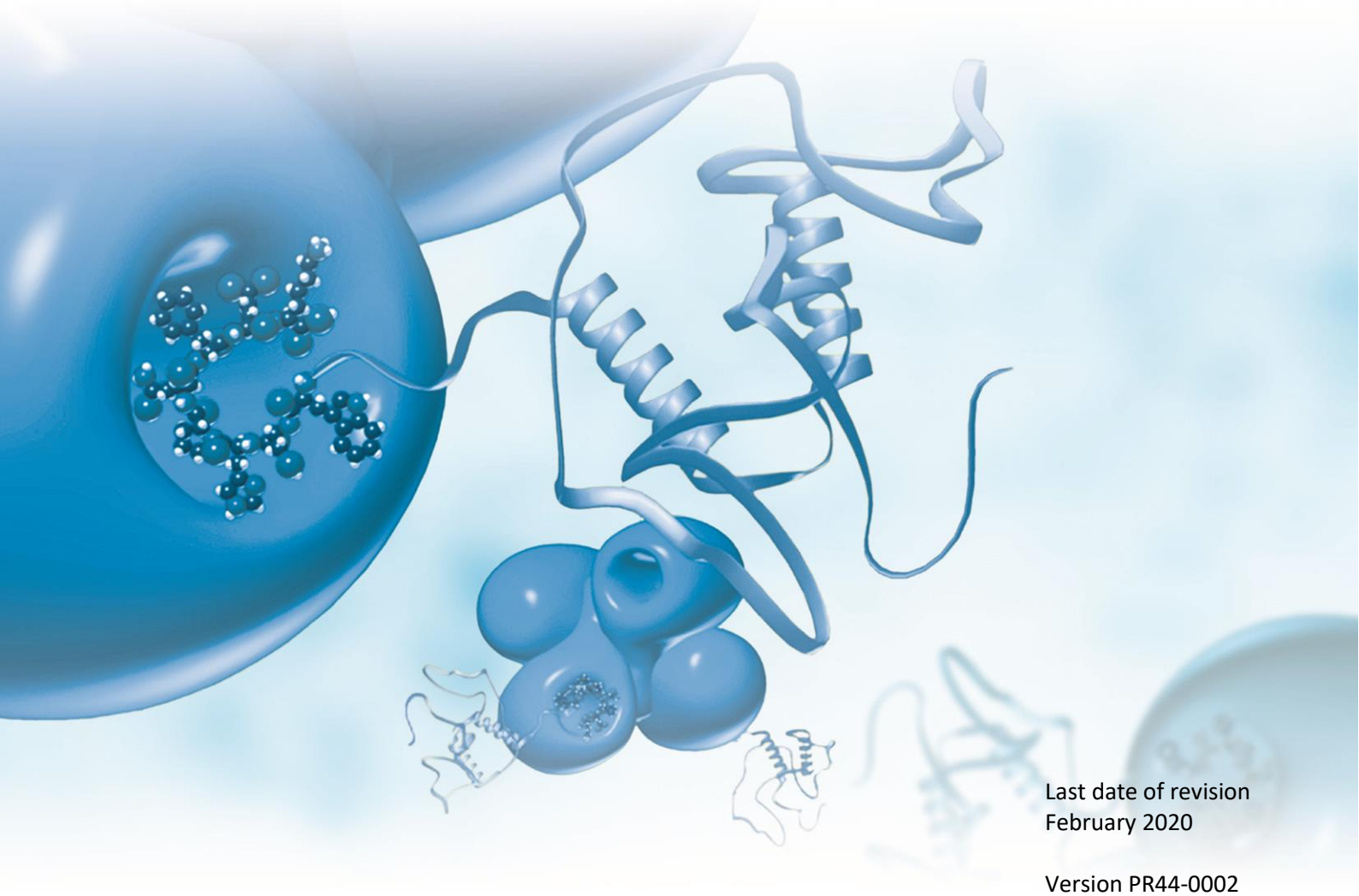
For Life Sciences

(Twin-) *Strep*-tag purification from cell culture supernatant with WET FRED (Flow REgulation Device)

Instruction protocol



Last date of revision
February 2020

Version PR44-0002

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Content

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1 Introduction

(Twin-)*Strep*-tag®/*Strep*-Tactin® affinity purification from cell culture supernatants with WET FRED

The *Strep*-tag® purification system is based on the highly selective binding of engineered streptavidin, called *Strep*-Tactin®, to *Strep*-tag® II fusion proteins. This technology allows one-step purification of almost any recombinant protein under physiological conditions, thus preserving its bioactivity. The *Strep*-tag® system can be used to purify functional *Strep*-tag® II proteins from any expression system including insect cells, mammalian cells, yeast and bacteria.
In mammalian and insect cell expression 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 (Twin-) *Strep*-tagged proteins the use of gravity flow columns is recommended over batch. This means a large volume has to be applied to a gravity flow column with a certain flow rate. To facilitate this step we have developed the WET FRED device. This device facilitates the transfer of large volumes, e.g. cell culture supernatants, to a *Strep*-Tactin® gravity flow column for purification of the recombinant target proteins fused to the (Twin-) *Strep*-tag. 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. Columns cannot run dry and need no supervision. Furthermore, no sophisticated software is needed facilitating set up and use.

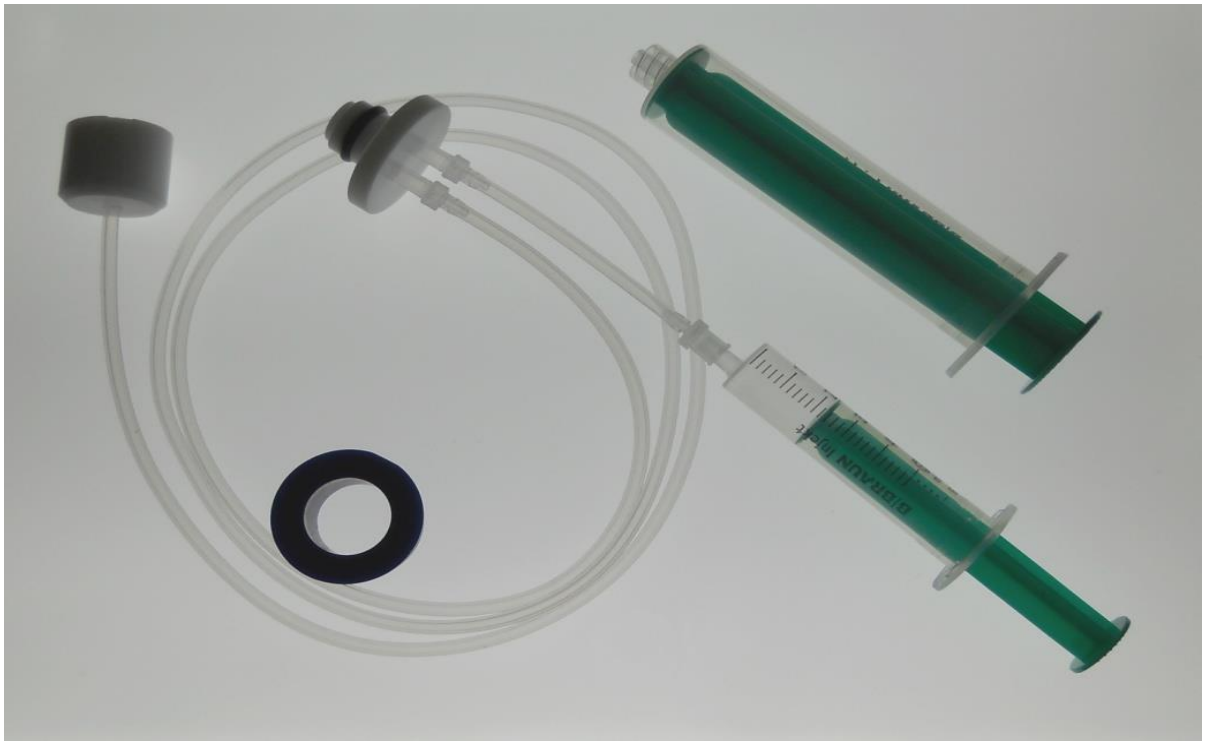

Fig.1 WET FRED Set-up

Single components of WET FRED

	1 ml column adapter		6 cm tube
	2x tube/luer adapter female		100 cm tube
	2x tube/luer adapter male		Bottle neck adapter for column
	20 ml syringe		Last drop filter
	5 ml syringe		

2 Protocol for affinity purification

Required Products/Buffers	Concentration of ingredients	Cat. No.
Buffer W (washing buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	
Buffer E (elution buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 2.5 mM desthiobiotin	
Buffer R (regeneration buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 1 mM HABA (hydroxy-azophenyl-benzoic acid)	
Gravity flow *Strep*-Tactin® Superflow high capacity column	1ml or 5ml	2-1209-001 or 2-1209-051
BioLock Solution	Activity : 70 U/ml	2-0205-050 or 2-0205-250
WET FRED	Device is re-usable after cleaning procedure	2-0910-001 or 2-0911-001

Important notes

- Please check the pH in the final cell supernatant before applying to the Strep-Tactin column. It should be above 7.0 to allow efficient *Strep*-tag® binding to *Strep*-Tactin®.
- Check the Biotin concentration of the used cell culture medium and add the appropriate amount of BioLock solution to prevent unspecific Biotin binding to *Strep*-Tactin®.
- The flow rate over the gravity flow column is an important factor for efficient binding of *Strep*-tag®. Please consider the given rates in the protocol.

Protocol
Sample preparation

1. **Remove the cells by centrifugation** (200 g) after cell culture.

2. **Add 0.1 volumes 10x buffer W** (e.g. for 1000ml culture 100ml buffer W (10x)) **and the necessary amount of BioLock solution** (information can be found under (www.iba-lifesciences.com >> technical-support >> View our FAQs and troubleshooting guides >> Biotin Blocking).
3. **Incubate for 15 min.**
   Clear supernatant by further centrifugation step (10,000 g).

4. **Apply the cleared supernatant to the gravity flow column**
   by using the WET FRED.

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Protocol
Assembly of WET FRED

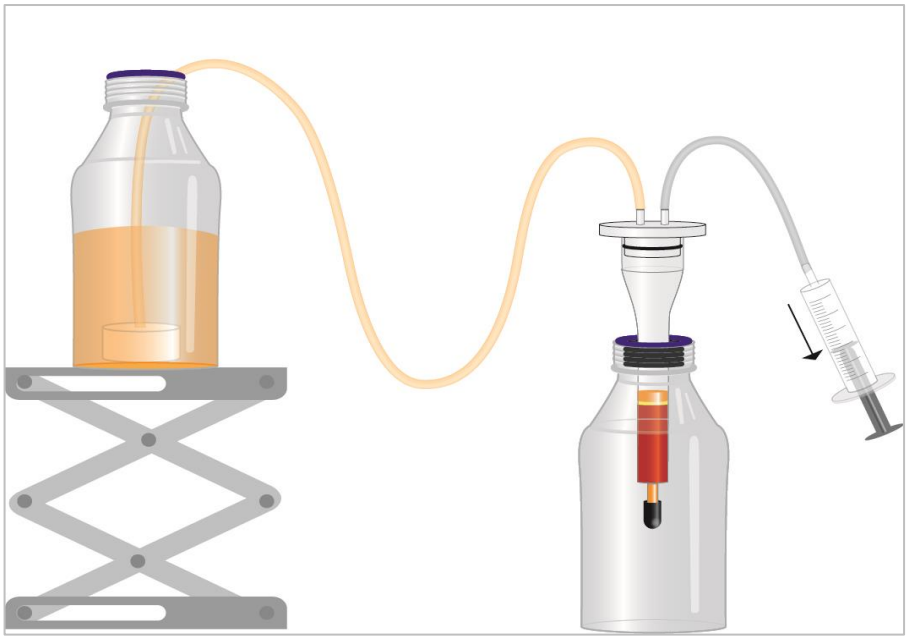

Fig.2

1. **Insert one end of the 100 cm tube into the last drop filter.**
   Wet one end of the tube with water to facilitate insertion.

2. **Use a *Strep*-Tactin column**,
   remove top and end cap, let drain the buffer in the column and close the column outlet with end cap.

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

4. **Gently plug the other end of the 100 cm tube to the column adapter via tube/luer adapter male.**

5. **Place the last drop filter on the bottom of the container**
   filled with the sample (culture supernatant).

6. **Gently plug 5 ml syringe via tube/luer adapter female to the 6 cm tube** and plug the other end of the tube (via adapter male) to the column adapter.

7. **Place the column via the bottle neck adapter into an appropriate bottle.**

8. **Gently aspirate (see arrow Fig.2) the sample with the 5 ml syringe** until the column bed is overlaid with 0.5-1 ml of sample.

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Protocol (continued)

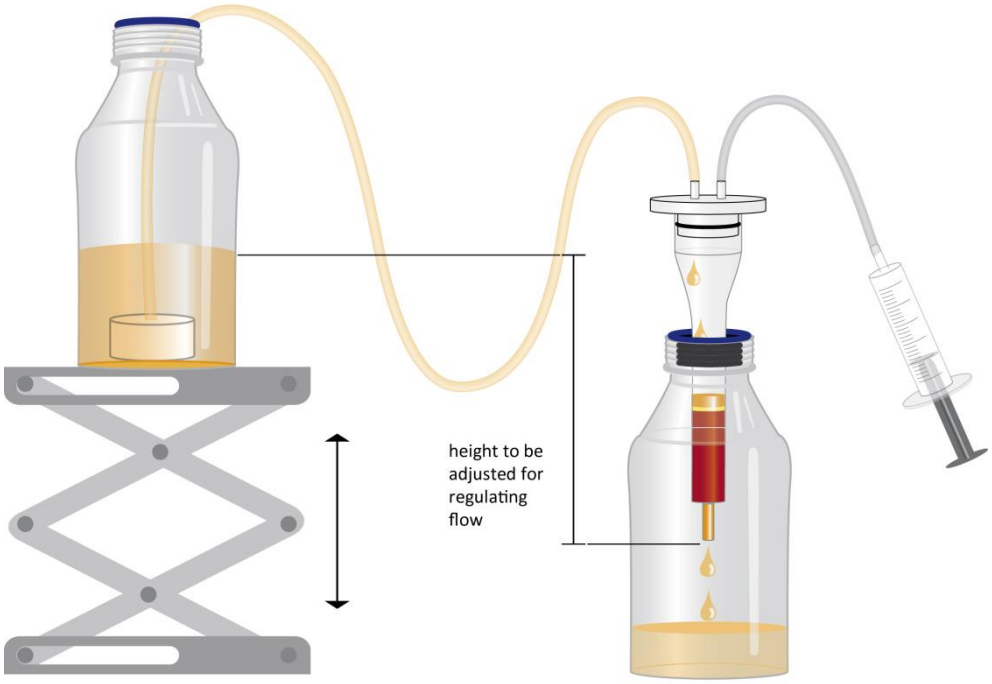

Fig.3

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

2. **Regulate flow to approximately 1 ml/min**
   by adapting height difference between column outlet and sample surface (see Fig 3). (20 drops equal approximately to 1 ml). 1 ml/min fits for all columns.
   In case of larger volumes, 5ml and 10ml columns can be run up to 3ml/min.

Important notes

15cm 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.

3. **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. (Column cannot run dry).
   Last drop filter ensures sample application to completeness and avoids clogging of the column.

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Protocol (continued)

After application of the whole sample WET FRED can be disconnected.

4. **First disconnect tubes from column adapter.**

5. **Then remove column adapter from column** and proceed to washing the column with 5x 1CV buffer W. Note, to use a new bottle to collect the flow-through.

6. **Followed by elution** with 6x 0.5CV buffer E in an appropriate vial.

Protocol
Cleaning of last drop filter, 100 cm tube and column adapter for re-use:

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

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

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.

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

5. **Clean column adapter by rinsing with water and ethanol.**

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

Please refer to **www.iba-lifesciences.com** for downloading this manual.

Your Notes

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