SEP 26, 2019

Protein purification strep-tag on gravity column

Cleo B.¹

¹Wageningen University

iGEM Wageningen 2019

Cleo B.

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.7qahmse

Protocol Citation: Cleo B. 2019. Protein purification strep-tag on gravity column . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7qahmse>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Sep 26, 2019

Last Modified: Sep 26, 2019

PROTOCOL integer ID:
 28130

Keywords: strep, protein, gravity, column

ABSTRACT

Protein purification of a protein with a strep-tag on a gravity column.

GUIDELINES

After lysis, sample should be kept on gel as much as possible

MATERIALS

MATERIALS

⊗ MilliQ water **Contributed by users**

⊗ Roche Complete Protease Inhibitor EDTA-Free tablets **Sigma Aldrich Catalog #5056489001**

50 % Strep-Tactinsepharose solution

BEFORE START INSTRUCTIONS

Have performed Protein expression using E. coli strain BL21DE3 by Robert Hooftman

buffers

- 1 Buffer W:
- 100 mM Tris-HCl (pH 8.0)
 - 150 mM NaCl
 - 1 mM EDTA

Buffer E:

- 100 mM Tris-HCl (pH 8.0)
- 150 mM NaCl
- 1 mM EDTA
- 2.5 mM biotin

Protein extraction

- 2 Place post-induction culture from Protein expression using E. coli strain BL21DE3 on ice
- 3 Centrifuge culture at 5000xg for 00:10:00 at 4 °C 1m
- 4 The pellet is resuspended in 1 mL buffer W per 100 mL cell culture containing one crushed cOmplete mini tablet.
- 5 The cells are sonificated (VS70 T rod, 25% 1 sec on 2 sec off for 00:05:00 . On ice water). For small amounts use the MS72 rod.
From this point be very sure to keep the cells on ice as much as possible.
- 6 The cell extract is centrifuged for 00:45:00 at 30 000 g. 4m
- 7 The supernatant is collected and filtered first with 0.45 um filter and then with 0,22 um filter. If there are small sample volumes: add 3 mL buffer W.

Protein purification

- 8 Fill centrifuge Column with 200-300 μL Strep- Tactinsepharose (so 400-600 μL 50% suspension). This is your CV.
- 9 Equilibrate with 3 Column bed Volume (CV) of Buffer W.
- 10 Load 10 CV of cell free extract on the column
- 11 Wash the resin 5 times by 1 CV of buffer W
Collect this in Eppendorf tubes per 1 CV. Apply 2 μL of the first washing fraction and 8 μL of each subsequent fraction to an analytical SDS-PAGE.
- 12 Apply 0.5 CV of Buffer E on column 6 times to elute the protein of interest
8 μL samples of each fraction can be used for SDS-PAGE analysis. Most of the purified Strep-tag® II fusion protein usually elutes in the 2nd to 5th fraction.
- 13 Determine protein concentration flow through on nanodrop with the molecular weight and extinction coefficient
- 14 Run on an appropriate SDS-page gel