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Protein purification strep-tag on gravity column

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We use this protocol and it's working

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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

Roche Complete Protease Inhibitor EDTA-Free tablets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**5056489001

50 % Strep-Tactinsepharose solution

Before start

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.

Protein extraction

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.

Protein extraction

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