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Protein Purification strep-tag FPLC

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

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Abstract

Protein purification of proteins containing a strep-tag using FPLC.

Necessary:
Have performed Protein expression using E. coli strain BL21DE3

To continue see: Protein gel sample preparation V2.

Materials

MATERIALS

MilliQ Water

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

Buffers

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

Filter this buffer with a 22 um filter

100 mL Buffer E:
- 100 mM Tris-HCl (pH 8.0)
- 150 mM NaCl
- 1 mM EDTA
- 2.5 mM desthiobiotin

1L 0.5 M NaOH (filtered with 22 um filter)

1L MQ (filtered with 22 um filter)

Protein extraction

2 Place post-induction culture from Protein expression using E. coli strain BL21DE3 on ice. For FPLC have 2 L of cell culture.

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 for 2 L cell culture.

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.

8

FPLC

9 Connect computer to system

10 Set a manual alarm specific for the column you're using
All speeds are dependent on the machine/column that is used

11 Wash all pumps with MQ

12 Wash the system with MQ (~20 mL)

13 Set valve position to waste

14 Connect column wet (MQ) with a low flow rate (~1 ml/min)
Wash column with MQ
Increase flow rate slowly

15 Equilibriate column with buffer W
Slowly increase flow rate
Wait until the conductivity and UV have stabilised

16 Wash peristaltic pump with 15 mL MQ
Introduce cell lysate in column using the peristaltic pump (connect wet)
Story flow through and a bit of lysed cells

17 Wash the column with buffer W until the lines stabilize

18 Start elution with buffer E
Collect all the fractions per 1/1.5 mL
Wash until the protein peak has passed

Place all the fractions on ice immediately
Fractions can be placed on analytical SDS-page gels

19 Wash the column with MQ (3 CV)
Wash the column with NaOH (3 CV)
Wash the column with ethanol (decrease speed before changing to ethanol) (3CV)
Wash the system with ethanol