Protein Purification with DEAE-Cellulose Anion-exchange Chromatography

Introduction

How to purify C-LytAm7 tagged proteins.

Materials

- 0.05M TRIS Maleic Buffer (pH 7)
  - 1 L
- 1M Maleic Solution
  - 20 mL
- 1.5 M NaCl Buffer
  - 400 mL
- 1.5 M Nacl + 2% Choline Buffer
  - 100 mL
- 1.5 M NaCl Solution
  - 200 mL

Procedure

Preparation for Initial Use

The columns are supplied in a storage buffer of 20% ethanol in water. The counter ion is OH^-. Prior to initial use and after extended storage periods, each column should be conditioned as described below (steps 1-4). Always use HPLC grade reagents and filter and degas buffers. During this operation do not exceed more than 25 % of the maximum recommended flow-rates.

1. Wash with 5 column volumes of dd. Water
2. Wash with 5 column volumes of 1.5M NaCl
3. Wash with 5 column volumes of low ionic strength equilibration buffer
4. Wash with 5 column volumes of high ionic strength limit buffer (1.5 M NaCl buffer)
5. Wash with 10 column volumes of low ionic strength equilibration buffer.

- The column may now be further equilibrated in the starting buffer at the desired flow rate.

DEAE-Sepharose Column Loading

1. Load 35 mL of your protein solution on the column
    a. Take 10 mL Fractions and measure protein presence ex. With biorad solution (should be purple colored)
2. Wash with 1.5M Nacl Buffer
    a. Take 10 mL Fractions and measure protein presence
    b. Wash step is done when there are no color change (no proteins)
3. Elute your Protein with 1.5M NaCl 2% Choline Buffer
    a. Take 10 mL Fraction and measure with biorad solution
    b. 2nd,3rd fraction should should be colored if the elution worked