Size Exclusion Chromatography Purification of Cas13a

Buffers

S200 Buffer:

10 mM HEPES (pH 7.5)

1 M NaCl

5 mM $MgCl_2$

2 mM DTT

Storage Buffer:

600 mM NaCl

50 mM Tris-HCl (pH 7.5)

5% v/v glycerol

2 mM DTT

Column Preparation

Purified using a Superdex75 large column from GE

1) Hook column up to AktaPrime chromatography system.
2) Wash column with at least 3 column volumes of MilliQ water (900 mL) at a rate of 1.5-2.0 mL per min.
3) Equilibrate column in 3 column volumes of S200 buffer (900 mL) at a rate of 1.5 mL per min.

Purification Procedure

1) Wash the loop with 10 mL of MilliQ water, followed by 10 mL of S200 buffer.
2) With system set to load, inject sample into the loop.

3) Set manual program to monitor UV at 215 nm, 254 nm, and 280 nm, and a 1 MPa pressure alarm. Switch to inject sample onto the column, and fractionate 2 mL fractions at a rate of 1.0 mL per min. Execute.
4) Collect fractions for about 250 mL, save fractions that have an A_{280} peak. Take a 50 µL sample for SDS PAGE analysis and store at 4 °C.
5) If another sample is being loaded onto the column, wash loop with 10 mL of buffer first before loading sample.
6) After purification(s) have finished, wash column with two column volumes (600 mL) of MilliQ water, followed by two column volumes of 20% ethanol (600 mL) for storage.

Concentration

1) Rinse Vivaspin MWCO 30000 with storage buffer (2 mL) and centrifuge at 4000 xg for 10 min
2) Pool elutions together.
3) Remove buffer from Vivaspin and add pooled elutions.
4) Centrifuge at 4000 xg for 5 min
5) Keep centrifuging: check for speed of concentrating. Concentrate to 1-2 mL. Watch for precipitate!
6) Remove filtrate from bottom of Vivaspin, and pool with other filtrate and store at 4 °C.
7) Aliquot protein sample and flash freeze in liquid nitrogen and store at -80 °C until needed.