Batch Affinity Purification of Cas13a

Protocol obtained from the Kothe lab. Buffer composition compared to Gootenberg *et al.*, 2017

Buffers

Buffer A:

20 mM Tris-HCl (pH 8.0)

400 mM KCl

5% v/v glycerol

30 mM Imidazole

1 mM βME

0.5 mM PMSF

Buffer B:

20 mM Tris-HCl (pH 8.0)

400 mM KCl

5% v/v glycerol

500 mM Imidazole

1 mM βME

S200 Buffer:

10 mM HEPES (pH 7.5)

1 M NaCl

5 mM $MgCl_2$

2 mM DTT

Preparation of Ni Sepharose Slurry

1) Gently shake resin bottle to create a homogenous medium and remove 5 mL slurry using a serological pipette. Transfer slurry to a 50 mL falcon tube, label with Ni-Sepharose, date, initials, and the protein
2) Spin the Ni-NTA sepharose column at 500 xg for 2 min to sediment the resin. Remove the supernatant.
3) Wash the resin with 3 volumes of sterile water. Gently mix to create a slurry. Spin at 500 xg for 5 min.
4) Wash the resin 6 times with Buffer A, leave 3 mL of Buffer A on the resin to obtain a 50% slurry.
5) Divide resin into more falcon tubes if lysate volume is greater than 50 mL.

Cell opening

1) Add PMSF and βME to buffer A
2) Resuspend the frozen cell pellet in buffer A (~5 mL/g of cell) in a small beaker. Thaw cells by stirring mixture slowly on ice (30-60 min).
3) Add lysozyme (1 mg/mL) and incubate the cell suspension on ice for 30 min
4) Add sodium deoxycholate (12.5 mg/g of cell). Continue stirring on ice for 30 min
5) Open cells using a sonicator while sample is on ice: at least 10 mins for 1 min, short pause in-between, shake beaker, intensity level 6, duty cycle at 60%
6) Centrifuge cell lysate for 30 mins at 30000 xg at 4 °C in a JA-25.50 rotor.
7) Take an A_{280} reading of cell lysate to monitor cell opening
8) Remove 50 µL of cell lysate for SDS-PAGE analysis
9) Store pellet for later analysis

Purification by Ni-Sepharose slurry

1) Apply clarified cell lysate to washed Ni-sepharose evenly and gently mix thoroughly. Incubate for 60 mins inverting periodically to bind protein to resin on ice.

   NOTE: all further steps are performed using cold buffers (4 °C).

2) Spin slurry at 500 xg for 5 min, remove the supernatant and store at 4 °C. Take a 50 µL sample for SDS PAGE analysis and store at 4 °C
3) Wash the resin 6 times with Buffer A, spinning at 500 xg for 5 min each time. Pool the washes, store at 4 °C. Take a 50 µL sample for SDS PAGE analysis and store at 4 °C.
4) Add βME to Buffer B.
5) Elute the protein 6 times using 90% resin volume of Buffer B. Incubate with buffer B for 5 min on ice. Spin for 5 min at 500 xg. Save elutions in 15 mL falcon tubes, store at 4 °C, and take a 50 µL sample for SDS PAGE analysis and store at 4 °C.
6) Run an SDS-PAGE of all samples at 180 V for ~3 hours.

Regeneration of Ni-sepharose

1) Wash resin 5 more times with 20 mL buffer B, centrifuge for 2 min at 500 xg. Check the last wash on a gel to check for protein presence.
2) Wash resin 3 times with 40 mL of d_2H_2O.
3) Wash resin once with 40 mL 20% ethanol. Leave 1.5 mL 20% ethanol on resin to obtain a 50% slurry for storage at 4 °C.

Concentration

1) Rinse Vivaspin MWCO 30000 with S200 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 5 mL. Watch for precipitate!

6) Remove filtrate from bottom of Vivaspin, and pool with other filtrate and store at 4 °C.
7) Store concentrated sample at 4 °C if not preceding directly to size exclusion chromatography.