AfriDX

COVID-19 diagnostics for Africa

Purification of Engineered Boon-scFv

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Preliminary note to user:

- The following protocol was designed for purifying Boon-scFv from OverExpress™ C43(DE3) competent *E. coli*
- A Thermo Scientific™ Pierce™ Protein Concentrators, PES 10K was used for concentrating and desalting the purified Boon-scFv

Materials:

- An induced *E. coli* cell pellet
- Lysozyme
- 1X PBS (pH 7.2)
- Triton X-100
- Protease inhibitor
- Tris(hydroxymethyl)aminomethane (TRIS)
- Sodium chloride (NaCl)
- Imidazole
- 8X Binding Buffer (4 M NaCl, 160 mM Tris-HCl, 40 mM imidazole, pH 7.9)
- 8X Wash Buffer (4 M NaCl, 480 mM imidazole, 160 mM Tris-HCl, pH 7.9)
- 4X Elute Buffer (4 M imidazole, 2 M NaCl, 80 mM Tris-HCl, pH 7.9)
- 4X Strip Buffer (2 M NaCl, 400 mM EDTA, 80 mM Tris-HCl, pH 7.9)
- 8X Charge Buffer (400 mM $NiSO_4$)
- Polypropylene chromatography columns
- Nickel NTA His•Bind Resin
- Microcentrifuge Tubes
- Pipettes
- Syringe (10 mL)
- Needle (27-28g)
- Thermo Scientific™ Pierce™ Protein Concentrators, PES 10K

Purification Protocol for Engineered Boon-scFv:

Cell Lysis:

Step 1: Suspend the cell pellet with 1 mg/mL of lysozyme in 1X PBS (pH 7.2). The final volume of the suspension is dependent on the volume of the bacteria culture used to form the pellet. For this protocol, a ratio of 1 mL of lysozyme per 10 mL *E. coli* culture is used.

Step 2: Homogenize the mixture gently and incubate for 30 – 45 mins while mixing at intervals of 10 mins. (Note: the reaction will gradually become viscous; attributable to DNA/RNA being released into solution as the cells undergo lysis. If the solution becomes too viscous to mix by gently shaking of the tube, you can skip the excess waiting time and proceed to the next step).

Step 3: Sonicate the lysis reaction (between medium-high amplitude) of about for 5 cycles (10 secs on; 5 secs off) and ensure that the preparation is placed on ice during the process. You may observe a significant reduction in viscosity after sonication. While the observations vary depending on the power of the sonicator's probe, it is advisable to shear excess DNA/RNA in the reaction as it may interfere with the separation of lysate from pellets and other downstream processes.

Step 4 (Optional): To shear excess DNA in the reaction, aspirate the sample with the syringe, insert the needle and expel into a tube. Repeat drawing and expelling through the needle until complete reduction in viscosity. Alternatively, use nucleases such as Benzonase to digest RNA and DNA in the lysis reaction.

Step 5: Allow the reaction to continue on ice for 30 minutes. Shake gently at intervals of 5 mins.

Step 6: After the lysis incubation, aliquot the cell lysis samples into microcentrifuge tubes.

Step 7: Centrifuge the lysis samples for 20 minutes at 13 krpm.

Step 8: Collect the cell lysate supernatant from the centrifuged samples. The solution should be pink in colour.

Purification:

Step 9: Mount polypropylene chromatography columns vertically on a support

Step 10: Completely suspended the His•Bind Resin by gently inversion of the bottle to mix.

Step 11: Transfer 1 mL of slurry to the column and allow the resin to pack under gravity.

Step 12: Remove the seal from the bottom of the column and allow the storage buffer to flow out.

Step 13: Charge and equilibrate the column in the following order

- *3 vol sterile deionized water*
- *5 vol 1X Charge Buffer*
- *3 vol 1X Binding Buffer*

[NOTE: 1 vol = 1 mL]

Step 14: Load column with prepared extract after the Binging buffer drains completely.

Step 15: Wash the column in the order

- *10 vol 1X Binding Buffer*
- *6 vol 1X Wash Buffer*

Step 16: Elute bound protein with 6 vol 1X Elute Buffer (you may capture the elute in fractions)

Step 17: Strip Ni 2+ from the column with 6 vol 1X Strip Buffer and store resin in 20% ethanol.

Desalting:

Step 18: Place the sample into the Concentrator sample chamber.

Step 19: Centrifuge the samples until the volume is reduced by 90–95%.

Step 20: Dilute the sample to the original volume with PBS.

Step 21: Repeat steps 2/3 **three** (3) times to buffer exchange of purified proteins into PBS.