AfriDX

COVID-19 diagnostics for Africa

Silica Purification of Engineered Boon-RTX

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Preliminary note to user:

- The following protocol was designed for purifying Boon-RTX from BL21 (DE3) cells
- For greater lysis efficiency, it is recommended that the probe sonication and heat purification steps are included.

Materials:

- An induced *E. coli* cell pellet
- Lysozyme
- 1X PBS (pH 7.2)
- 1X PBS (pH 7.5)
- 1X RTX Storage Buffer (pH 8.0)- 50 mM Tris-HCl, 50 mM KCl, 25 % glycerol, 0.1 % Tween-20.
- Microcentrifuge Tubes
- Centrifuge Tubes
- Glass Beaker
- Duran Bottle
- Syringe
- 0.2 µm Filter
- Pipettes
- 60µm silica gel
- Magnetic Stir Bar

Protein Purification Protocol for Engineered Boon-RTX:

Step 1: Suspend the cell pellet with 1 mg/mL of lysozyme in 1X PBS (pH 7.5). 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: Incubate the lysis solution at room temperature for 30 minutes.

Step 3 (Optional): Keeping the lysis solution on ice, sonicate the sample using 40% amplitude for 4 minutes. The sonicator should be set to 1 sec ON/ 4 sec OFF. It should be noted that a 40% amplitude for a ½" diameter probe is approximately 48µm.

Step 4 (Optional): Centrifuge the lysis solution for 30 minutes at 3750 rpm. Pre-heat water in a glass beaker to approximately 70 degrees. The beaker should be large enough to place a Duran bottle inside.

Step 5 (Optional): Transfer the cell lysis supernatant to a Duran bottle and place this into the glass beaker at 70 degrees for 10 minutes. A magnetic stir bar can be used to allow uniform heat dissipation, or alternatively, swirl the solution every 30 seconds.

Step 6 (Optional): Transfer the heat-treated solution to a centrifuge tube and centrifuge for 30 minutes at 3750 rpm.

Step 7 (Optional): Carefully transfer the heat-treated supernatant to a syringe fitted with a 0.2 µm filter. Filter the solution into microcentrifuge tubes. The solution should be pink in colour.

Step 8: If the optional steps were not performed, then after the lysis incubation, aliquot the cell lysis samples into microcentrifuge tubes and centrifuge for 30 minutes at 13 krpm. After centrifugation the supernatant should be collected, the solution should be pink in colour. If the optional steps were performed, ignore this step.

Step 9: Prepare a solution of 60µm silica gel with a concentration of 25 mg/mL using 1X PBS (pH 7.2). It should be noted that vortexing and inverting the tube/container can assist in suspending the silica gel.

Step 10: Prepare the silica for immobilisation by first preparing microcentrifuge tubes with 20 mg of 60µm silica gel (0.8 mL from the stock prepared in **Step 9**).

Step 11: Centrifuge the prepared silica at 7.5 krpm for 5 minutes.

Step 12: After centrifugation, remove the supernatant, leaving only silica gel in the tube.

Step 13: Add the cell lysate directly to the silica, at a ratio of 1 mL of cell lysate to 20 mg of silica gel.

Step 11: Suspend the silica in the cell lysate using a vortexer, and afterwards lay the tube flat on its side to spread out the settling microparticles.

Step 12: Incubate the silica with the cell lysate for 30 minutes at room temperature. To ensure even absorption across the silica microparticles, vortex the sample every 10 minutes.

Step 13: After silica immobilisation, first vortex the sample and then centrifuge at 7.5 krpm for 5 minutes.

Step 14: Remove the supernatant, leaving behind silica with immobilised Boon-RTX. The silica itself should be visibly pink, while the supernatant is colourless.

Step 15: Perform 2 rounds of washing on the silica immobilised Boon-RTX in RTX storage buffer (pH 8.0).

Washing Steps:

- Add 1 mL of 1X RTX storage buffer (pH 8.0)
- Suspend the immobilised protein with a vortex
- Centrifuge the protein at 7.5 krpm for 5 minutes
- Remove the “wash” buffer

Step 16: Suspend the immobilised Boon-RTX in 1X RTX storage buffer (pH 8.0) to its working concentration. A ratio of 5µL of buffer to 1 mg of silica gel is used.

Step 17: Collect and combine the immobilised Boon-RTX. The working stock can be stored at -20°C both short and long term.