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COVID-19 diagnostics for Africa

Silica Purification of Engineered Boon2-BST

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Preliminary note to user:

- The following protocol was designed for purifying Boon2-BST from BL21 (DE3) cells
- For greater lysis efficiency, probe sonication can be used.

Materials:

- An induced *E. coli* cell pellet
- Lysozyme
- 1X PBS (pH 7.2)
- **Tris(hydroxymethyl)aminomethane (TRIS)**
- **Potassium chloride (KCl)**
- **Ethylenediaminetetraacetic acid (EDTA)**
- **Dithiothreitol (DTT)**
- **Triton X-100**
- **BST Storage Buffer (10mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA, 0.1% Triton X-100, pH 7.1)**
- Microcentrifuge Tubes
- Pipettes
- 60µm silica gel

Protein Purification Protocol for Engineered Boon2-BST:

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: Incubate the lysis solution at room temperature for 30 minutes.

Step 3: 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. In addition, this solution can be prepared and set aside during the lysis incubation step.

Step 4: After the lysis incubation, aliquot the cell lysis samples into microcentrifuge tubes.

Step 5: Centrifuge the lysis samples for 30 minutes at 13 krpm.

Step 6: Collect the cell lysate supernatant from the centrifuged samples. The solution should be pink in colour.

Step 7: 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 3**).

Step 8: Centrifuge the prepared silica at 7.5 krpm for 5 minutes.

Step 9: After centrifugation, remove the supernatant, leaving only silica gel in the tube.

Step 10: 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 Boon2-BST. The silica itself should be visibly pink, while the supernatant is colourless.

Step 15: Perform **4** rounds of washing on the silica immobilised Boon2-BST.

Washing Steps:

- Add 1 mL of **BST Storage Buffer (pH 7.1)**
- Suspend the immobilised protein with a vortex
- Centrifuge the protein at 7.5 krpm for 5 minutes
- Removes the “wash” buffer

Step 16: Suspend the immobilised Boon2-BST in **BST Storage Buffer (pH 7.1)** 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 Boon2-BST. The working stock can be stored at 4°C both short and long term.