AfriDX

COVID-19 diagnostics for Africa

Protein Expression of Engineered Boon2-BST

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Preliminary note to user:

- The following protocol was designed using BL21 (DE3) as the host *E. coli* strain
- All the steps should be performed under sterile conditions until step 13 (with the exception of the incubation steps)

Materials:

- Conical Flask
- Bunsen Burner or Biosafety cabinet (for environment sterility)
- LB Broth
- Centrifuge Tubes
- Pipettes
- Kanamycin
- Isopropyl β-d-1-thiogalactopyranoside (IPTG)
- Glycerol stock of BL21 (DE3) transformed with the pET24a – Boon2-BST plasmid

Protein Expression Protocol of Engineered Boon2-BST:

The protein expression process starts with an overnight culture.

Step 1: Using 70% ethanol, sterilise the benchtop/biosafety cabinet to be used.

Step 2: Using 70% ethanol, sterilise and clean any pipettes that are to be used.

Step 3: Add 5mL of LB Broth to a sterile centrifuge tube.

Step 4: Add kanamycin to the LB Broth for a final concentration of 50 µg/mL. (I.e. if the stock concentration of the kanamycin is 100 mg/mL, add 2.5 µL of the stock to 5 mL of LB Broth.)

$$\frac{50\ \frac{\mu g}{mL} \times 5\ mL}{100\ \frac{mg}{mL}} = 2.5\ \mu L$$

Step 5: Inoculate the prepared LB Broth with 1 µL of the Boon2-BST bacterial glycerol stock.

Step 6: Incubate the culture overnight (12 to 16 hours) at 37 °C and 225 rpm.

The following steps are performed the next morning.

Step 7: Add the desired culture volume worth of fresh LB Broth to a sterile conical flask (I.e. 50 mL, 100 mL, 200 mL, etc). The maximum volume is dictated by the volume of the flask. Standard protocols utilise an air-to-volume ratio of 10:1. This means that if a 2 L flask is being used, the max desired volume of LB Broth should be 200 mL.

Step 8: Add kanamycin to the LB Broth for a final concentration of 50 µg/mL. (I.e. if the stock concentration of the kanamycin is 100 mg/mL and the desired culture volume is 200 mL, add 100 µL of the stock to the LB Broth.)

$$\frac{50\ \frac{\mu g}{mL} \times 200\ mL}{100\ \frac{mg}{mL}} = 100\ \mu L$$

Step 9: Inoculate the prepared culture flask with the overnight culture.

Step 10: Incubate the large culture at 37 °C and 225 rpm until an OD_{600} of 0.6 to 0.8 is reached.

Step 11: Once an appropriate OD_{600} has been reached, induce protein production by adding IPTG for a final concentration of 1 mM. (I.e. if the stock concentration of the IPTG is 0.8 M and the culture volume is 200 mL, add 250 µL of the stock to the culture.)

$$\frac{1\ mM \times 200\ mL}{0.8\ M} = 250\ \mu L$$

Step 12: Incubate the induced culture at 37 °C and 225 rpm for 4.5 hours.

Step 13: Aliquot the culture equally into centrifuge tubes.

Step 14: Centrifuge the culture at 3750 rpm for 30 minutes.

Step 15: Decant the supernatant. The pellets can be stored at -20 °C long term or 4 °C short term.