

## AfriDX

COVID-19 diagnostics for Africa

# Protein Expression of Engineered Boon-RTX

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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  $\beta$ -d-1-thiogalactopyranoside (IPTG)
- Glycerol stock of BL21 (DE3) transformed with the pET24a-Boon-RTX

### Protein Expression Protocol of Engineered Boon-RTX:

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 5 mL of LB Broth to a sterile centrifuge tube.

**Step 4:** Add kanamycin to the LB Broth for a final concentration of 50  $\mu\text{g}/\text{mL}$ . (I.e. if the stock concentration of the kanamycin is 100  $\text{mg}/\text{mL}$ , add 2.5  $\mu\text{L}$  of the stock to 5 mL of LB Broth.)

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

**Step 5:** Inoculate the prepared LB Broth with 1  $\mu\text{L}$  of the Boon-RTX 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.)

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

**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<sub>600</sub> of 0.6 to 0.8 is reached.

**Step 11:** Once an appropriate OD<sub>600</sub> 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.)

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

**Step 12:** Incubate the induced culture at 18 °C and 225 rpm for 18 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.