AfriDX

COVID-19 diagnostics for Africa

Autoinduction Protein Expression of Engineered Fluorescent Single Chained Antibody

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Preliminary note to user:

- The following protocol was designed using OverExpress™ C43(DE3) competent cells as the host *E. coli* strain
- All the steps should be performed under sterile conditions until step 11 (with the exception of the incubation steps)

Materials:

- Sodium phosphate dibasic (Na_2HPO_4)
- Potassium phosphate monobasic (KH_2PO_4)
- Sodium chloride (NaCl)
- Tryptone
- Yeast extract
- Glycerol
- Glucose
- Lactose
- Water
- LB Broth
- Bunsen Burner or Biosafety cabinet (for environment sterility)
- 2 autoclavable 1 litre bottle
- 3 autoclavable 250 mL bottles
- Conical Flask
- Centrifuge Tubes
- Pipettes
- Kanamycin
- Glycerol stock of OverExpress™ C43(DE3) transformed with the pSANG10 – scFv-mCh plasmid

Prepare autoinduction media:

Prepare the following for autoclaving.

Solution 1 (2x Media): Weigh out and add the following to a 1 litre autoclavable bottle:

- 6 g of Na_2HPO_4
- 3 g of KH_2PO_4
- 5 g NaCl
- 20 g of Triptone
- 5 g Yeast Extract
- 500 mL of water

Solution 2 (50% vol/vol glycerol): Pour the following into a 250 mL autoclavable bottle:

- 50 mL of glycerol
- 50 mL of water

Solution 3 (10% weight/vol glucose): Add/Pour the following into a 250 mL autoclavable bottle:

- 5 g of glucose
- 100 mL of water

Solution 4 (5% weight/vol lactose): Add/Pour the following into a 250 mL autoclavable bottle:

- 10 g of lactose
- 200 mL of water

Solution 5: Pour at least 500 mL of water into a 1 L autoclavable bottle.

Autoclave all 5 solutions.

Reconstitute autoinduction media: Under sterile conditions add the following to the sterilised 2x media (solution 1):

- 12 mL of sterile 50% glycerol
- 5 mL of sterile 10% glucose
- 40 mL of sterile 5% lactose
- Top up to 1 L using sterile water

Protein Expression Protocol of Engineered fluorescent fused single chained antibody:

Day-1 (Overnight/Starter Culture)

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 and supplement with 0.2% (w/v) of glucose (ie: Add 100 µL of 10% glucose. Note that although addition of glucose at this stage is not compulsory, it is recommended for C43 strains in order to minimise expression of target proteins prior to induction).

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 scFv-mCh bacterial glycerol stock (or use a single colony from a transformation experiment if C43 cells were freshly transformed with plasmids).

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

Day-2 (Protein Expression)

The following steps are performed the next morning.

Step 7: Add the desired culture volume worth of autoinduction media 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. (The volume used in this experiment is 200mL)

Step 8: Add kanamycin to the autoinduction media 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 at a ratio of 100:1. (I.e. if the desired culture volume is 200 mL, add 2 mL of the overnight culture.)

Step 10: Incubate the large culture at 37 °C and 225 rpm for about 1 hour and then monitor the culture until the OD (optical density) reaches between 0.8 -1.0 or 0.8±0.05.

Step 11: At the desired OD, transfer the cultures to 25 °C with shaking at 225 rpm for 18-24 hours.

Step 12: Aliquot the culture equally into centrifuge tubes.

Step 13: Centrifuge the culture at 3750 rpm and 4 °C for 30 minutes.

Step 13: Decant the supernatant. The pellets can be stored at -20 °C long term or 4 °C short term.