Look What They've Done To My Shoes!

SCU_China Project 2016

Protocol

VHb Team

Look What They've Done To My Shoes!

Protocol for VHb SDS-PAGE.

1. Cultivate bacteria for 10h at 37C°, 300rpm.
2. Centrifugate bacteria at 10000xg 5min.
3. Discard the supernatant, add 20ml PBS.
4. Use high pressure cell disrupters to lyse the bacteria.
5. Centrifuge the lysate at 30000xg, 4C° for 30min.
6. Separate the supernatant and precipitate.
7. (Alternative) Supernatant condense: add 800ul ethanol to 200ul supernatant in -20C° staying for 5min, centrifuge it at 10000xg 4C° for 1min. Repeat the step and add proper volume of PBS to finish the condense.
8. Add 30ul loading buffer to precipitate(or the whole bacteria) and add 5ul loading buffer to 25ul supernatant, 95C° heating for 5min.
9. Prepare the 15% SDS Running Gel solution.
10. Prepare 5% SDS Stacking Running Gel solution.
11. Sample added and 5ul protein maker added
12. 200V Electrophoresis till the blue band to the bottom
13. Add gel into warm coomassie brilliant blue G250 for 2h
14. Add gel into warm destaining solution till the bands are clear.

15% 10ml SDS Running Gel solution.
Water 2.3ml
30% Acrylamide 5.0ml
1.5M Tris-HCl, pH8.8 2.5ml
10% SDS 0.1ml
10% APS 0.1ml
TEMED 0.004ml

5% 5ml SDS Stacking Running Gel solution
Water 2.74ml
30% Acrylamide 0.66ml
1.5M Tris-HCl, pH6.8 0.5ml
10% SDS 0.04ml
10% APS 0.04ml
TEMED 0.004ml

Loading Buffer Composition:
10% w/v SDS
10 mM Dithiothreitol, or beta-mercapto-ethanol
20 % v/v Glycerol
0.2 M Tris-HCl, pH 6.8
0.05% w/v Bromophenolblue

Protocol for Oxygen Concentration and Gradient Oxygen Concentration Cultivation

1. Use foam sponge to make two scaffold which can be loaded with 2 50ml tube in MGC C2500G box.
2. Use 75% ethanol to disinfect the box and sponge.
3. Put the whole device in UV light at least 15min.
4. Load 50ml tubes in the hole of sponge and add the oxygen pack and close the device in the biology safe cabinet.
5. Use ropes to bind the device tightly in shaker. The shaking speed can't beyond 200rpm/min.

Protocol for Gradient Oxygen Concentration Cultivation Preparation.

1. Take out 1.5ml culture with bacteria of J04450 and K1919500 to test OD600.
2. Calculate and take out proper culture with bacteria of J04450 and K1919500 to dilute the OD600 into 0.2.
3. Add 2ul of OD600=0.2 bacteria into new liquid culture to make sure the same volume of bacteria at the beginning of cultivation.