GeneCopoeia™
Expressway to Discovery

Efficient Biotinylation E. coli B Strain AVB101

Catalog：AVB101

Size： 1 vial, containing 0.5 mL of glycerol stock

Shipping and storage conditions： Ship on dry ice and store immediately at –80 °C

Description

Strain AVB101 is an E. coli B strain (hsdR, lon11, su1A1), containing a pACYC184 plasmid with an IPTG-inducible birA gene to overexpress biotin ligase (pBirAcm). This strain is recommended for protein expression because of its robust growth and the absence of the OmpT and Lon proteases.

Cultivation and Induction

TYH Media, 1 Liter :

20 g Tryptone
10 g Yeast extract
11 g HEPES
5 g NaCl
1 g $MgSO_4$
adjust to pH 7.2-7.4 with KOH

1. Grow a 10 ml overnight culture from a single colony or glycerol stock in TYH media supplemented with 10 µg/ml chloramphenicol and the appropriate antibiotic (eg. ampicillin) needed to maintain the expression vector with shaking at 37℃.
2. Place 5 ml of the overnight into 1 L of TYH media in a baffled Fernbach flask with 100 µg/ml ampicillin.
   Note: Chloramphenicol is not included.
3. Add 20 ml of a 20% sterile glucose solution (0.5% final conc.) and shake vigorously at 37℃.
4. When the OD_{600} of the mixture reaches 0.7, remove 1.5 ml as a pre-induction sample.
5. Add 10 ml of 5 mM biotin solution (50 µm final). The biotin solution is made by adding 12 mg of d-biotin to 10 ml of warm (microwaved) 10 mM bicine buffer (pH 8.3) and filter-sterilizing the solution with a syringe and a 0.2 micron filter.
6. Add 15 ml of 100 mM IPTG (1.5 mM IPTG final) to induce for 3 hr.
7. Pellet cells in 4 x 250 ml centrifuge bottles at 5858 x g for 10 min.
8. Pour off media from cell pellets, re-suspend each pellet in 10 ml B-PER (Pierce Chemical Company, Pittsburgh, PA) (40 ml total volume).
9. Shake on a rotary shaker 10 min, RT.
10. Combine suspensions into one bottle and centrifuge at 16,270 x g for 15 min.
11. Save supernatant. Re-suspend pellet in 25 ml B-PER. Shake on a rotary shaker 10 min, RT.
12. Centrifuge at 16,270 x g for 15 min.
13. Add supernatant to that previously saved. Discard pellet.

Pre-induction and induced samples of bacterial proteins can be analyzed by SDS-PAGE, western blotting with labeled Streptavidin, or enzymatic means.

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