Cultivation and Induction of AVB 101

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 ^{o}C.
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 ^{o}C.
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.