www.avidity.com | phone: 720-859-6111 | info@avidity.com

Sample Induction Protocol for pAN/pAC/BIP-300 AviTag™ fusion constructs in AVB101

Materials

-TYH Liquid Culture Media: 1 Liter

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

-20% glucose solution
-Ampicillin
-Chloramphenicol
-B-PER II Bacterial Protein Extraction Reagent (Thermo Scientific/Pierce Chemical Co.)

Procedure:

1. Start a 10mL overnight culture, preferably from a single colony, in TYH liquid culture media supplemented with 10µg/mL chloramphenicol for maintenance of the BirA over-expression plasmid of the AVB101 and the appropriate antibiotic (e.g. ampicillin for the pAN/pAC/BIP-300 plasmid vectors) needed to maintain the expression vector with shaking for aeration at 37°C.
2. Add 5mL of the overnight growth culture into 1L of TYH media/100µg/mL ampicillin in a baffled 2.8L Fernbach flask. Note: Chloramphenicol is not added for pACYC-184 maintenance any longer (see the FAQs section for explanation).
3. Add 20mL of a 20% sterile glucose solution (0.5% final conc.) and shake vigorously at 37°C.
4. When the OD_{600} of the culture reaches 0.7AU, remove 1.5mL as a pre-induction sample.
5. Add 10ml of 5mM d-biotin solution (50µm final). The biotin solution is made by adding 12mg of d-biotin to 10ml of warm 10mM bicine buffer (pH 8.3) and filter-sterilized with a syringe and a 0.2 micron filter.
6. Add 15ml of 100mM IPTG solution (1.5mM final) and induce for 3 hours at 37°C (or lower if desired).
7. Pellet the cells in four 250mL centrifuge bottles spun at 6000 x g for 10-15 min.
8. Pour off the media from cell pellets and re-suspend each pellet in 10mL B-PER II for 40mL of total lysis suspension volume.
9. Shake on a rotary shaker or otherwise gently agitate for 10 minutes at room temperature.
10. Combine the lysis suspensions into one bottle and centrifuge at 16,000 x g for 15 min.
11. Pour off and save the spin supernatant. Re-suspend pellet in 25ml B-PER II. Again, shake on a rotary shaker 10 min, RT.
12. Centrifuge at 16,000 x g for 15 min.
13. Add the spin 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.