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Phage screening - colorimetric test

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We use this protocol and it's working

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Abstract

Phage Screening - Colorimetric Test protocol involves a series of steps designed to detect the presence of specific bacteriophages through a colorimetric assay. This comprehensive procedure aims to provide a robust method for screening and assessing bacteriophage activity within microbial cultures, essential for various research and diagnostic applications.

Attachments

Colorimetric_Phage_a...

822KB

Materials

1. 15ml Falcon tube
2. 0.5% sodium hypochlorite
3. 70% ethanol
4. Cotton/Tissue paper
5. Gloves
6. Tube holder
7. Discard bag
8. Permanent marker
9. 0.22µm syringe filter
10. 5ml Syringe
11. LB broth
12. Pipette sets
13. Sterile tips - 200µl 1000µl
14. Disposable Serological Pipets, Sterile, 25 mL

Protocol materials

50ml AlamarBlue Cell Viability Assay Reagent **G-Biosciences Catalog #**786-923 Step 10

Day 1

1 Prepare LB-agar plates.

2 Prepare LB-broth.

3 To recover bacteria from stock open the tube and use a sterile loop, toothpick or pipette tip to scrap off some bacteria and streak on appropriate media plates using the overnight culture and grow at 37 °C .

4 If using lyophilized strain skip step 3 and 5 and go to step #6.1

Day 2

5 Next, start overnight cultures by inoculating fresh liquid media (5 mL) with a single colony and
grown for 14-16 hours at 37 °C in a shaker incubator.

Note

Note: Make sure you are using only one colony to start the experiment.

Day 3

6 Inoculate 5 mL of fresh LB (1% inoculum) and grow at 37 °C until the cells reach a density of OD_{600}=0.2. The amount of host culture to add is not critical, but it should be enough that would normally produce a saturated culture over the period of the enrichment (0.2 OD is enough or 0.5 McFarland Standard i.e. 1.5x 10^8 CFU/ml).

Note

If using lyophilized strain go to step #6.1

6.1 Using a pipette, aseptically add 500 µL of the recommended growth medium to the freeze-dried material and mix well.

6.2 Transfer the entire suspension to a test tube containing 5 mL of the recommended media.

7 In a new falcon tube add 1 mL of filtered water sample to 900 µL of LB-broth and inoculate with 100 µL of a fresh host culture (0.2 OD) and mix gently.

Note

If using lyophilized strain take 100 µL of host culture previously diluted in step 6.2.

7.1 In another falcon tube inoculate a positive control adding 10-50uL (this amount will depend of the concentration of your phage stock) of phage stock to 1900 µL of LB-broth with 100 µL of a fresh host culture and mix gently.

8 Incubate this enrichment culture at 37 °C for at least 2 hours (or under whatever conditions the host favors). At the same time inoculate a new tube with 5 mL of fresh LB (1% inoculum) and grow at 37 °C for 2 hours.

9 Filter the culture through a 0.22µm syringe filter. The filtrate is now ready for testing.

10 In a new falcon tube add 100 µL of sample (filtrate from previous step) to 1800 µL of LB-broth and inoculate with 100 µL of a fresh host culture (0.2 OD) and add 50 µL

of 50ml AlamarBlue Cell Viability Assay Reagent **G-Biosciences Catalog #**786-923 (resazourin) and mix gently.

11 Incubate the culture at 37 °C for 3 hours. Check the culture every hour to monitor color change.

12 If the sample is positive for phage culture will remain blue. If the sample is negative culture will change to pink color.