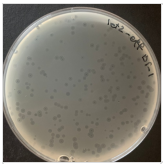

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Enumeration and Propagation of Bacteriophage MS-2

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DISCLAIMER

ABSTRACT

This Protocol guides through setp-by-step enumeration and propagation of MS-2 bacteriophage. It is prepared and adjusted for ***Escherichia coli*(ATCC 15597) strain C-3000**and**MS-2 phage (ATCC-15597-B1).**

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Stock Preparation

1

1.1 LB Broth Dilution Tubes

1. Add 20 g of LB broth to 1 liter of milli-Q
2. Heat and stir until warm and fully dissolved
3. Autoclave at 121°C for 20 minutes
4. Aseptically transfer 900 µL of sterile broth to 1.5 mL microcentrifuge tubes

1.2 E.coli (ATCC 15597) Medium preparation – Medium 271

1. Add 20 g of LB broth to / mL of DI water
2. Heat and stir until warm and fully dissolved
3. Autoclave at 121 C for 20 minutes.
4. Let it cool down to 50C in water bath.
5. Add 50 mL of SOL B aseptically.

SOL A- LB
SOL B – 1
Glucose 10.0 g, Cacl2 2.94g, Thiamine 0.1 g (0.0005M), DI water 500 mL – filter sterilized using 0.22 µm filter in a sterilized vessel

SOL B-2
Thiamine HCL:**3.73 gr in 10 mL of water (1M)**
Then add**0.3 mL to 500 mL of Solution B-1**

SOL B: SOL B-1+SOL B-2

1.3 1.1Fresh Host Cell Stock: Escherichia coli strain B- using flasks 95 mL LB and 5 mL inoculation

1. At the end of the day (3pm) add 5 mL of*E.coli*B stock to 95 ml of medium (LB broth+ SOLB)
2. The following morning (at 9 am) dilute overnight stock 100 times in fresh medium (LB broth+ SOLB)
3. Incubate for 3hrs and 15 min to get to the mid-exponential phaseat 37°C and**200 rpm**
4. Add 1.5 mL of**mid-exponential stock**with 500 µL of 50%**glycerol**(sterilized) in 2mL labeled cryotube
5. Store at -80°C until needed.

1.4 Bacteriophage MS-2 Stock preparation

1. Add 1 mL stock*E. coli*B into**9**mL of medium (LB broth+SOLB) and incubate for Mid exponential phase 3 hrs and 15 min (**37°C**and**200 rpm)**
2. Add**500**µL of bacteriophage MS-2 stock and 1 mL of sterilized 0.2 M $CaCl_2$– [2.9402 g/ 100mL].
3. Incubate overnight (**20**-24 hr) at**37°C**and**200 rpm.**
4. Cells and debris should be removed from the phage lysate by centrifugation at (3000 RPM) 3500 RPM for 15 min at room temperature.
5. The phage-containing supernatant should be filter 0.45 µm- using sterilized syringe filters- into fresh sterilized falcon tubes.

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6. Stock solution should be near 10^{10}-10^{11}PFU/mL.
7. Store at 4°C for 1-2 months.

1.5 Creating Stock from the vial

1. Add 1 mL stock *E. coli* B into **9** mL of LB broth and incubate for Mid exponential phase 3 hrs and 15 min (**37°C** and **200 rpm).**
2. Open the ampule and suspend freeze dried powder using (1.5 mL) sterile PBS; make sure is fully mixed. Transfer aliquots of 500 µL to sterile microcentrifuge tubes. They stay active for at least two months at 4 °C.
3. Add **100** µL of bacteriophage MS-2 stock and 1 mL of sterilized 0.2 M $CaCl_2$– [2.9402 g/ 100mL].
4. Incubate overnight (20-24 hr) at **37°C** and **200 rpm.**
5. Cells and debris should be removed from the phage lysate by centrifugation at (3000 RPM) 3500 RPM for 10 min at room temperature.
6. Recover stock by filtering at 0.45 µm- using sterilized syringe filters into fresh sterilized falcon tubes.
7. Stock solution should be near 10^{10}-10^{11}PFU/mL.
8. Store at 4°C for 1-2 months.

Agar Plate preparation

2

1. Prepare 450 mL – Part A bottom and upper layer agars as per Table 2.1. Autoclave at 121°C for 20 minutes.
2. After autoclaving, let it cool down using the water bath at between 45-50C
3. Add the Part B solution aseptically. SOL B is heat sensitive and higher temperature may cause precipitation of $CaCl_2$ or disintegration of thiamin HCL.
4. Add **10 mL** of bottom layer to Petri dish (100mm*15mm) and allow to harden. You should get ~50 plates. Make sure leaving the lid partially open not to get condensations, **Condensation causes inaccuracy**.
5. Temper upper layer at range of 45-50°C, do not add SOL B if you are not ready to use it.

- If you are using in small portions, then

Use a 50 mL falcon tube, add 40 mL of top layer and 4 mL of SOL B. Vortex briefly.

- If you are using the whole 450 mL,
- Then add 50 mL of SOL B

	Component	Bottom Layer (1% agar)	Upper Layer (0.9% agar)
Part A	**LB Broth**	10 g	10 g
Bacto Agar	5 g	4 g	
Milli-Q	450 mL	450 mL	
Part B	**Sol B**	50	50

Table1- Top and Bottom Layer Agar composition

MS-2 Enumeration

3 Prepare *E.coli* B solution by incubating 1 mL of *E.coli* B stock in 9 mL medium (LB broth+ SOL B).

- Incubate overnight for 16-18 hrs.
- Incubate for until in mid-exponential phase (3 hours and 15 min).
- After incubation is done add 1mL of sterilized $CaCl_2$ **[brings the final Con to 20 mM]- quick vortex to mix it up.**

1. Serial dilution of MS-2 in LB using 1.5 mL microcentrifuge tubes (900 µL PBS + 100 µL of MS-2).
2. Label plates based on the serial dilution, date and name (duplicates).
3. Label fresh, sterile 15 mL tubes as serial dilution (10-1, 10-2, ….10-9)- These are for next step of adding *E. coli.*
4. Add 500 µL of prepared *E.coli* to the labelled 15 mL tubes, then add 100 µL of serially diluted MS-2 and 100 µL

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of $CaCl_2$ Solution to the corresponding *E. coli* tubes – incubate for **20 minutes** at **room temperature** to allow for attachment of MS-2 to bacteria and before replication.

5. Add 3 mL of upper layer to 15 mL falcon tubes (mixture of MS-2 and *E. coli*)
6. mix it using your hand or vortex- avoid any bubbles.
7. Pour it on the bottom layer plates and swirl it until fully spread.
8. Allow for upper layer to harden.
9. Keep control plate- just *E. coli* and no dilution of MS-2.
10. Incubate at 37°C for 18-24 hr.
11. Count Plaques.

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