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Phage stock preparation

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Works for me

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ABSTRACT

A protocol for phage lambda and phage T7 phage stock preparation

- 1 Grow E. coli bacterial host (for example LE392, DH10B or DH5alpha) in LB medium overnight at 37°C
- 2 Prepare and autoclave 1 Molarity (M) CaCl_2 and 1 Molarity (M) MgCl_2
- 3 Add $50\text{ }\mu\text{l}$ of 1 Molarity (M) CaCl_2 and $50\text{ }\mu\text{l}$ of 1 Molarity (M) MgCl_2 to 50 ml of LB medium. Make aliquots of 10 mL and inoculate with 0.1 volumes of overnight bacterial host.
- 4 Incubate with agitation for $01:00:00$ at 37°C .
- 5 Add $100\text{ }\mu\text{l}$ of high titer phage lysate ($> 10^8$ PFU/ml).
- 6 Incubate at 37°C with agitation for $\sim 05:00:00$ or until lysate clears.
- 7 Collect phage lysate and store at 4°C until clean up.
- 8 Pool phage lysate into 50 ml sterile falcon centrifuge tubes and centrifuge at 4,000 rpm for $00:25:00$.
- 9 Filter-sterilize the phage supernatant using a $0.22\text{ }\mu\text{m}$ filter to yield a bacterial cell-free phage lysate.
- 10 Determine phage titer using [plaque](#) or [spot](#) assay.



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