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# Plaque assay

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1 Works for me dx.doi.org/10.17504/protocols.io.7i8hkhw

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ABSTRACT

This is a protocol for the quantification of phage lamba and T7 titers by counting plaques.

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Liquid LB medium | | |
| LB agar | View | |

1 Streak LB plate with E. coli (strain should susceptible to phage lambda. Strains that include a prophage lambda are likely to be resistant. Strains LE392 and DH10B have been used with this protocol) and incubate overnight at **37 °C**

2 Pick a colony from this plate and use it to inoculate **10 ml** of LB. Incubate this culture at **37 °C** until OD reaches 2-3. Overnight culture is recommended.

3 Make a range of dilutions . Mix **100 µl** of phage dilution with **200 µl** of E. coli culture. Then incubate for 10 minutes at room temperature. After **00:10:00** add **3 ml** of liquid soft LB agar (LB agar with 0.7% agar) ( **50 °C** ).

4 Pour the resulting mixture on LB plates (preheat plates at **37 °C** ). Spread the mixture on the plate by moving it. Make sure to work quickly to avoid clumps of solidified agar.

5 Incubate for **00:15:00** at room temperature. Afterwards turn the plates over and incubate overnight at **37 °C** .

6 Use plates with 30-300 plaques to determine phage concentration. Calculate the Plaque Forming Units (PFU)/mL by the following formula: PFU/mL = $N \times 1/DF \times 1/V$.
N is the number of plaques of lysis counted on the plate (expressed as PFU); DF is the dilution factor and V is the volume of phage dilution poured on the plate.