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Viral purification from bacterial culture

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for the purification of viral particles from bacterial liquid culture

Materials

50 ml Falcon tube, 0.45 µm syringe filter & syringe, SM buffer, DNase I, 10x DNase buffer , heatblock, 20 % SDS , Proteinase K, phenol:chloroform:isoamyl , phase lock gel light tubes , TE buffer



- 1 Centrifuge 40 ml of bacterial culture infected with phage at 6000 g for 30 min, remove the supernatant and filter it through a 0.45 µm syringe filter
- 2 Centrifuge the filtrate at 35 000 g for 4 h, remove the supernatant and re-suspend the pellet in 600 µl SM buffer
- 3 Add 2 µl of DNase I and 20 µl of 10x DNase buffer and incubate at 37°C for 1.5 h
- 4 Incubate sample at 65°C for 30 min to inactivate DNase I
- 5 Add 10 µl of 20 % SDS and 40 µl of Proteinase K (20 mg/ ml) and incubate at 37°C for 1 h
- 6 After the incubation, mix the sample with an equal amount of phenol:chloroform:isoamyl pH 8.0 (25:24:1) alcohol in a phase lock gel light tubes and centrifuge at 12 000 g for 5 min
- 7 Add 600 µl more of the phenol:chloroform:isoamyl alcohol to the tube and centrifuge at 12 000 g for 5 min
- 8 Transfer the aqueous phase to a new tube and add 1200 µl cold 100% ethanol
- 9 Incubate sample overnight at -80°C, and then centrifuge at 16 000 g for at 4°C for 1 h
- 10 Remove the supernatant and re-suspend the pellet in 100 µl TE buffer
- 11 Store at 4°C