# Viral purification from bacterial culture

sarah.schulz[1,2]

[1]EMBL; [2]NFDI4Microbiota

sarah.schulz

MAR 16, 2023

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.e6nvwjq7wlmk/v1

**Protocol Citation:** sarah.schulz 2023. Viral purification from bacterial culture. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwjq7wlmk/v1



**Protocol status:** Working
We use this protocol and it's working

**Created:** Mar 06, 2023

**Last Modified:** Mar 16, 2023

**PROTOCOL integer ID:** 78214

**Keywords:** phage purification, phage

## 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