DNA Extraction from Bacteriophages - 96 well format

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Extraction of viral DNA/RNA from culture lysate or filtered samples free from bacteria. The protocol is based on the Qiagen kit QIAamp Viral RNA Mini kit for 96 well plates and uses consumables from this kit as well as the Pierce Universal Nuclease.

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Before beginning this protocol, ensure that wash buffers have been properly diluted with ethanol and that the centrifuge is available and not chilled at low temperatures may impede ethanol evaporation.

1 Pipet **1 µL** 100x diluted Pierce Universal Nuclease (2.5units/uL) to each well of a Qiagen S-block

2 Transfer **140 µL** of each sample from the outer chamber of the CentrisArt filter tube to the S-block. Mix by pipetting

3 Incubate for **00:05:00** at room temperature 5m

4 Add **540 µL** AVL buffer to inactivate nucleases and lyse phage heads. Mix by pipetting

5 Incubate **00:10:00** at room temperature 10m

6 Add **560 µL** absolute ethanol. Mix thoroughly by pipetting

7 Place a QIAamp 96 plate on a new S-block

8 Transfer **630 µL** to the QIAamp 96 plate, then seal it with an AirPore tape sheet

9 Load the QIAamp 96 plate on the S-block into a rotor bucket. Centrifuge **5500 x g, 21°C, 00:04:00** or until all liquid has passed through the filter 4m

10 Repeat steps 8 and 9 until all of the sample has passed through the filter

11 Replace the bottom S-block and add **500 µL** AW1 wash buffer to each well, then seal the QIAamp 96 plate

12 Load the QIAamp 96 plate on the S-block into a rotor bucket. Centrifuge 5m

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5500 x g, 21°C, 00:05:00 or until all liquid has passed through the filter

13 Remove the seal, add **500 µL** AW2 wash buffer to each well, then reseal the plate.

14 Load the QIAamp 96 plate on the S-block into a rotor bucket. Centrifuge **5500 x g, 21°C, 00:05:00** or until all liquid has passed through the filter 5m

15 Place the QIAamp 96 plate on a new S-block and centrifuge **5500 x g, 21°C, 00:10:00** to dry the membrane 10m

16 Discard the S-block and place the QIAamp 96 plate on an A&A receiving plate. Add **60 µL** AVE elution buffer directly onto the filter membrane

17 Seal the plate with an AirPore Tape sheet, then incubate at room temperature for **00:10:00** 10m

18 Load the QIAamp 96 plate on the receiving plate into a rotor bucket. Centrifuge **5500 x g, 21°C, 00:04:00** 4m

19 Discard the QIAamp 96 plate, seal the receiving plate for storage and store at **-80 °C**

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