# Sampling for extracellular phage quantification by qPCR and MPN assays Version 4

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## Abstract

*Sampling is for qPCR assay to determine total extracellular phage (qEXT), and for MPN (most probable number) assay to determine infective phage concentration. Samples containing phage and cells are filtered through a 0.2µm membrane in 96-well format to remove cells; filtrate fraction is saved for later analysis.*

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## Before start

***Check the filter plates carefully for cracks and chips. We frequently receive plates that have been damaged in shipping. If the plate is cracked or damaged, you risk losing your samples in the centrifugation step.***

## Materials

- 96-well filter plates (0.2um) Millipore #MSGVN2210 by Contributed by users
- 96 well plates for filtrate Fisher #087722C by Contributed by users
- Eppendorf centrifuge with plate adapters and a balance plate by Contributed by users
- P1000 micropipet and Filter Tips by Contributed by users
- Parafilm by Contributed by users

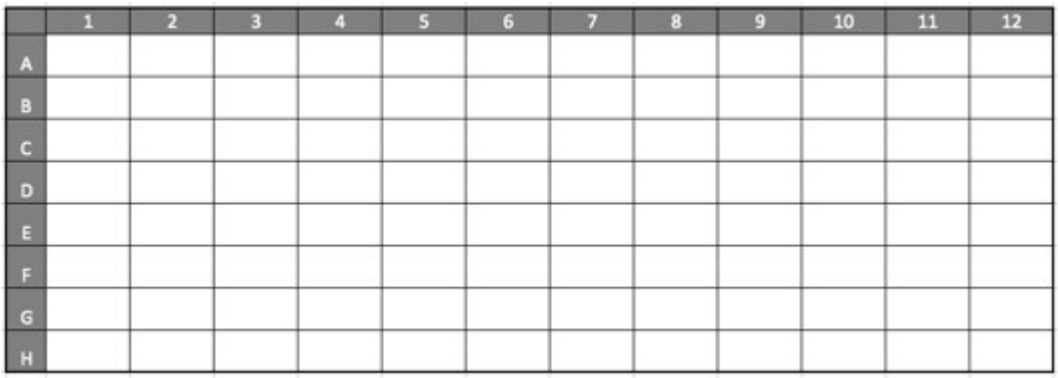
## Protocol

### Step 1.

Place filter plate (MSGVN2210) onto receiver 96-well plate without touching the bottom of the sterile filter plate.

Keep a log of your sample layout (which samples are in which wells).

**Plate-Setup Template:**



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

**Step 2.**

Transfer 250 µL of sample from each experimental bottle/tube into empty wells on the filter plate in duplicate. It is not necessary to fill the entire plate; we will continue to use the same plate over the course of the experiment.

**Step 3.**

Centrifuge at 1000 x g for 3 min. Be sure to balance the centrifuge.

**Step 4.**

Between sampling, store stacked filter/filtrate plates at 4°C, covered with a plate lid. Do not disturb the stacked plates, to avoid cross-contamination between wells.

**Step 5.**

After filling the entire plate, remove the filter plate and discard. Store the filtrate plate at 4°C, covered with a plate lid and wrapped well with parafilm, until analysis.