VIRAPREP

Concentration of coliphages and bacteriophages

- Illustrated protocol -

FIRST STEP : MATERIAL

Kit content

- 100 membranes, 0.22µm, 47mm,
- 2 bottles of Binding Buffer (400ml),
- 2 bottles of Elution Buffer (500ml),
- 100 sterile tubes,
- Operating mode.

Additional material

- Filtering manifold and vacuum pump,
- Sterile funnels, tweezers and scissors,
- Pipette and tips 1ml,
- Ultrasonic bath,
- Vortex.

To safely store the Elution Buffer over a long period of time, we recommend you to aliquot it into the sterile tubes supplied, and store them in the fridge. Before beginning the analysis, take the required number of tubes out of the fridge. This protocol is in accordance with the standards EN ISO 10705-1, 10705-2 and 10705-3.

SECOND STREP : SAMPLE PREPARATION

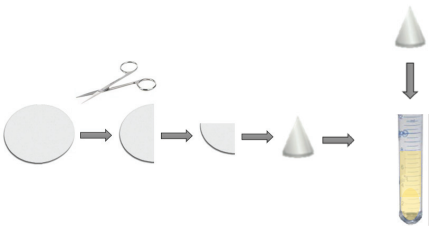
1. Add the Binding Buffer 50X in the sample to be analysed respecting the following proportions: 2 ml of Binding Buffer for 100 ml of water sample.

2. Sterilize each filter holder of the manifold with flame and alcohol. Let them cool down a few minutes before placing the membranes on top.

THIRD STEP : SAMPLE PROCESSING

3. Place the sterile funnels (plastic or stainless steel) on the filter holders. Pour the sample containing the Binding Buffer into the funnels.

4. Open the valves and start the filtration. To ensure an optimal retention, apply a vacuum of 0.6 to 0.8bar.



5. When filtration is over, close the valves. Cut the membranes in 8 parts using steril scissors. Place them in the tube containing 5 ml of Elution Buffer.

6. Place the tubes in an ultrasonic bath 4 min*. Then vortex 10 sec, 2000 RPM. Perform plate assay using 1ml to 5ml of sample according to ISO 10705-2**.

*If you do not have an ultrasonic bath, you can use a vortes. Optimal conditions: 4min, 37°C, 1000 RPM.
**Use an agar concentration of 6 g/l for the solid media. Let the petri dish dry before incubation.

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