Millipore®
Preparation, Separation,
Filtration & Monitoring Products

MERCK

Concentration of coliphages
How-to-Use Guide

1. 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 & vacuum pump,
- Sterile funnels, tweezers & scissors,
- Pipette & 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.

2. STEP: SAMPLE PREPARATION

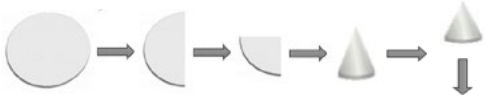
1. Add the Binding Buffer 50X in the sample to be analysed respecting the following proportions: 2 ml of Binding Buffer for 100 ml of water sample.

2. Sterilize each filter holder of the manifold.

3. STEP: SAMPLE PROCESSING

3. Pour the sample containing the Binding buffer into a filtration manifold.

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 thermoshaker 4 min, 37°C, 1000 RPM, then vortex 10 sec.
**Use an agar concentration of 6 g/l for the solid media. Let the petri dish dry before incubation.

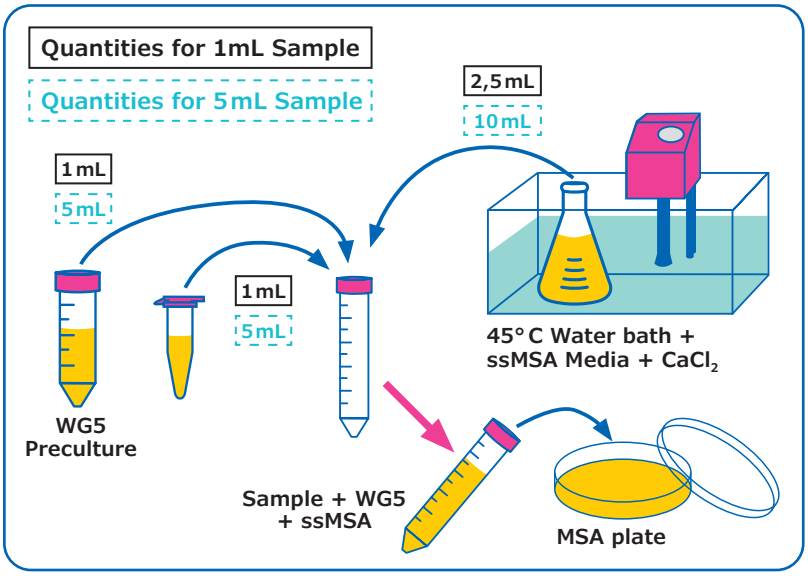
Culture protocol according to ISO 10705-2

Prepare the bacterial culture and the culture media MSA and ssMSA according to standard ISO 10705-2.

1. Remove the MSA plates from the fridge, allow to warm at room temperature.
2. Melt the ssMSA and place it in a water bath at 45 ± 1°C.
3. Add 6 µl/ml of CaCl2 (14.6%) solution to the ssMSA.
4. Mix the components respecting the following proportions:

	For 1 ml sample	For 5 ml sample	Membrane culture
Bacterial preculture	1 ml	1 ml	1 ml
ssMSA + CaCl2	2,5 ml	10 ml	2,5 ml

5. Pour the mixture on the MSA plates. **Step 4 and 5 must be done quickly to obtain a homogenous layer and avoid premature solidification of the ssMSA.**
6. In parallel, place the membrane pieces face down onthe MSA inoculated with the bacterial suspension only.
7. Let the plates dry opened for 10 min.
8. Incubate for 18h at 37°C. Count the number of plaques.



Ref	Origin	Designation
5248-10x100ML	CondaLab	ss-MSA, bottle 100 mL in 125 mL bottle (x10)
5247-10x200mL	CondaLab	MSA, Bottle 200mL in 250 mL bottle (x10)
5249	CondaLab	MSB, bottle 25mL (x10) in 125 mL bottle (x10)
AR00206 VIRAPREP Kit	GLBiocontrol	VIRAPREP Somatic coliphages concentration kit
BINDING-1	GLBiocontrol	BINDING BUFFER
PHIX174	GLBiocontrol	CONTROL POSITIVE
Z769584		Branson M series ultrasonic bath
Z742555		MicroBead Sterilizer with glass beads
746495		Calcium chloride
N8878		Nalidixic acid

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