



The quickest, most accurate and easy-to-use viral indicator tests available for **microbial water quality assessment**

Analysis of Somatic Coliphages Rapid Kit for Presence/Absence in 100 ml

Application

Drinking water, reclaimed water and other samples with expected low counts of somatic coliphages

User Guide

A NEW APPROACH
FOR WATER TESTING



50 assays

Cat. No. BPF-SPA

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1. DESCRIPTION AND KIT PRINCIPLE

Bacteriophages as viral indicators have emerged to improve quality controls of water, biosolids and food. They provide complementary advantages to bacterial indicators since they are present in a similar way but usually persist longer in the environment and provide information about viral pathogens which are not properly represented by studying only bacterial indicators.

Issues such as resuscitation or recovery of injured bacteriophages do not seem to occur. This is an advantage when clear effects of the treatment process need to be evaluated and certified.

Somatic coliphages are bacteriophages of enteric origin that infect *Escherichia coli* through cell surface receptors. They are

detected by lysis of suitable bacteria strains (host bacteria). The presence of somatic coliphages in a water sample usually indicates pollution by human or animal faeces or by wastewater containing these excreta. Their resistance in water and food resemble that of human enteric viruses more closely than faecal bacteria, commonly used as water or food quality indicators.

This Presence/Absence Kit is designed for the specific detection of somatic coliphages in 100 mL of water. It is based on the colour change that occurs in the culture medium due to the lysis of the host strain if the somatic coliphages are present in the water sample (Patent US 9,932,645 B2 and EPO 3068894), and a catalysed reaction by beta-glucuronidase. The kit contains materials for performing 50 assays.

2. KIT CONTENTS

	Reagent/Material	Storage	Description
50 x 	<i>E.coli</i> CB10 Host Strain Vial	(-20±2)°C	Screw-capped plastic vial containing the <i>E.coli</i> CB10 host strain and PBS for resuspension
50 x 	Bluephage Testing Bottle	Room temperature	BP Testing Bottle containing the Bluephage medium for analysis of 100 mL water samples
10 x 	Bacteriophage φX174	(-20±2)°C	Screw-capped plastic vial containing lyophilised positive control and PBS for resuspension

3. EQUIPMENT NOT INCLUDED

- Incubator thermostatically controlled at (36±2)°C or water bath set at (36±2)°C.
- Incubator thermostatically controlled at (30±2)°C.

4. ASSAY TIME TO RESULTS

The time to results is 6 hours when starting the BP Testing Bottle incubation.

5. GENERAL RECOMMENDATIONS

It is important to maintain aseptic conditions during the procedure to avoid cross contamination between samples and contamination from external sources.

This kit has been designed to analyze samples with predicted counts of somatic coliphages between 0 and 1000 PFU/100 mL. Dilute samples if higher counts are expected.

The maximum turbidity permitted in samples is 100 NTU.

For marine water, dilute samples 1/4 using distilled water.

6. QUALITY CONTROL

It is always recommended to analyze replicates for each sample.

Negative Control. It is always recommended in each series of samples to examine a procedural blank using sterile diluent as the sample.

Positive Control. It is always recommended in each series of samples to examine a reference control of φX174 bacteriophage. Materials for up to ten positive controls are supplied in this kit.

- Prewarm the lyophilised positive control at room temperature.
- Remove the seal from the cap and gently rotate it clockwise to release the reference bacteriophage.
- Shake gently to resuspend the lyophilised bacteriophage and let it stand for 10 minutes upside-down at room temperature.
- Mix the 3.5 mL resuspended with sterile water or commercial mineral water until 100 mL is available.
- Proceed with Section 7 as any other sample.

7. PROCEDURE

1. Preparing the sample



Prewarm the water sample at $(36 \pm 2)^\circ\text{C}$. It is very important that the sample reaches this temperature before starting the analysis.

2. Preparing the host strain



Prewarm the Host Strain Vial at room temperature. Remove the seal from the cap and gently rotate it clockwise to release the host strain. Shake vigorously to resuspend the host strain and let it stand for 10 minutes upside-down at room temperature.

3. Water sample



Measure 100 mL of the water sample. The 100 mL mark is just an orientation reference. Open the BP Testing Bottle and pour in the water sample.

4. Mixing the resuspended host strain and the sample



Open the Host Strain Vial and check that all the contents in the cap have been resuspended. Pour the host strain into the BP Testing Bottle. Close the bottle.

5. Starting the test. Resuspension of Bluephage Medium.



Press the top of the BP Testing Bottle cap to make the Bluephage Medium fall inside the bottle. Shake gently to completely dissolve the Bluephage Medium in the water sample. Do not turn the bottle up and down.

6. Running the test



Incubate the BP Testing Bottle at $(36 \pm 2)^\circ\text{C}$ during 1 h. Then, transfer the BP Testing Bottle to an incubator at $(30 \pm 2)^\circ\text{C}$ during 5 h more. Total incubation time is 6 h.

7. Reading the results



Read the test results after 5h. If the yellow colour is maintained (negative), it indicates absence of somatic coliphages. If colour changes to green-blue (positive), there is presence of somatic coliphages. The colour change always occurs before 6h if there is a presence of bacteriophages, even at very low concentrations ($1 \text{ PFU} / 100 \text{ mL}$). Do not extend the incubation and reading of tests beyond the time when the negative control presents a start of colour change due to enter in the natural death phase of the culture. In case of confirmation of negative results, see Spot Test at Section 8.

8. SPOT TEST

This kit is designed to detect 1 PFU/100 mL within 6 h. For optimal reliability, negative results may be confirmed by spot test:

1. After the 6 h incubation, open the BP Testing Bottle containing Negative Control (see Section 6) and introduce a sterile swab.
2. Extend the inoculum from the swab in a Modified Scholten's Agar plate (Cat. No. BP1638) assuring that the entire surface is covered.
3. Open the BP Testing Bottle containing the sample to be confirmed and put three drops (10 µL approx.) in the inoculated plate.

4. Incubate upside-up at (36±2)°C for (18±2)h.

5. Examine the plate and record sample as positive for somatic coliphage presence if a clear lysis zone appear in the place where any of the three drops were put.

9. DISPOSAL OF USED MATERIALS

Safe disposal of used materials should be done by users as a matter of good practices and industry standards and should conform to national/international environmental, health and safety regulations.

10. TROUBLESHOOTING

Problem	Possible cause	Suggestions
Negative control is positive.	<ol style="list-style-type: none">1. There is cross-contamination with a positive sample.2. The test has exceeded 6 hours since its starting. The culture of the host strain has begun the death phase of growth kinetics.	<ol style="list-style-type: none">1. The results obtained should be discarded and the samples retested, strictly following the instructions provided in this manual. Do not reuse any reagents or bottles.2. Always avoid extending the test beyond the 6 hours indicated.
Positive control is negative.	<ol style="list-style-type: none">1. Something has gone wrong in the analysis.2. The stock of bacteriophage is degraded.	<p>The results obtained should be discarded and the samples retested.</p> <ol style="list-style-type: none">1. Strictly follow the instructions provided in this manual.2. Strictly follow the rehydration and storage protocols for bacteriophage stock explained in Section 6. Discard the current used bacteriophage stock and rehydrate a new one.
After the 6 h incubation, no colour change is detected and the turbidity in the BP Testing Bottle is low.	Sample contains many somatic coliphages (>1000 PFU/100 mL) and the host strain could not grow.	Dilute the original sample and repeat the analysis.

11. RELATED PRODUCTS

Positive control for the Enumeration of Somatic Coliphages. Usable with the Rapid Kits for the analysis of somatic or total coliphages. φX174 Reference bacteriophage (Cat. No. BPF-pX174).

12. LEGAL NOTICE

Product warranty

This product has been designed for enumeration of somatic coliphages, and its performance is guaranteed in the manner described in this brochure. The purchaser must determine the suitability of the product for its particular use. Bluephage rejects any implicit warranty for any other use or adaptation to particular purposes. No other licence is granted expressly, impliedly, or by estoppel.

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