



# ***Pseudoalteromonas espejiana* bacteriophage PM2**

**27025-B1<sup>TM</sup>**

## **Description**

*Pseudoalteromonas espejiana* bacteriophage PM2 is a bacteriophage that was isolated from seawater. This virus requires the host *Pseudoalteromonas espejiana* BAL 31 (ATCC 27025) for propagation.

**Strain designation:** PM2

**Deposited As:** PM2

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## **Storage Conditions**

**Product format:** Frozen

**Storage conditions:** -80°C or colder

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## **Intended Use**

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

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## **BSL 1**

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local



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or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

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## **Certificate of Analysis**

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## **Growth Conditions**

**Host:** *Pseudoalteromonas espejiana* BAL 31 (ATCC 27025)

**Medium:**

ATCC Medium 588: AMS medium

**Temperature:** 30°C

**Atmosphere:** Aerobic

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## **Handling Procedures**

1. Follow general procedures given below for phage propagation.
2. Use *Pseudoalteromonas espejiana* BAL 31 (ATCC 27025) as host.



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## GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

To recover phage from freeze-dried or thawed frozen vial:

1. Prepare an actively growing broth culture of the recommended host strain 18-24 hours before opening the phage specimen. The host should be in early log phase.
2. Melt several tubes of soft agar (0.5% agar) and maintain at 45-55°C until ready to use.
3. Prewarm plates of the recommended medium in an incubator for approximately 15-30 minutes prior to the plating of the bacteriophage.
4. Add 100  $\mu$ L of the host culture to a pre-melted 0.5% agar tube and pipette gently to evenly mix, avoiding the creation of bubbles.
  - a. Overlay the agar plate surface with 2.5 -3 mL of melted 0.5% agar containing the host. Allow overlay to harden.
5. Perform the broth serial dilution in quadruplicate using a 96 well plate.
  - a. Dispense 90  $\mu$ L of broth medium into each well.
  - b. Add 10  $\mu$ L of phage lysate to each of the four  $10^{-1}$  wells. Mix thoroughly by pipetting up and down at least 15 times.
  - c. Transfer 10  $\mu$ L from the  $10^{-1}$  wells to the  $10^{-2}$  wells and mix. Continue the serial dilution until at least  $10^{-8}$ .
  - d. Using a multichannel pipette, spot 2  $\mu$ L of each dilution on the agar overlay. Allow to dry before moving the plates to the appropriate incubator
6. Incubate plates agar side down at the appropriate temperature. Incubation times will vary depending on the phage. Most only need 12-18 hours. Incubating for too long can make the plaques very difficult to read.

To propagate phage:

1. Open the host organism according to the information on the product sheet.
2. Pick one colony from the isolation plate and homogenize in 5 mL of the appropriate broth.
  - a. Note: It may be necessary to inoculate a larger volume or more test tubes based on the volume needed for the next amplification.
3. Incubate at the appropriate temperature in a shaking incubator until it reaches OD<sub>600</sub> of 0.1 – 0.3.
4. Thaw or rehydrate the bacteriophage vial. Use 0.5 mL of the appropriate broth

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to rehydrate freeze-dried material.

- a. Infect each 5 mL culture with 100  $\mu$ L of the bacteriophage and shake at the appropriate temperature overnight. Prepare a fresh subculture of the host.
- b. Centrifuge phage culture at 4000  $\times$  g for 10 minutes.
- c. Filter the lysate with a 0.2  $\mu$ m PES sterile filter and store the filtrate at 4°C.
- d. Perform a spot titer as per the instructions above.
- e. They may also be frozen with or without cryoprotectant. If available, liquid nitrogen storage is the best method for long term storage. Most phage can also be freeze-dried. We use double-strength skim milk mixed half-and-half with the filtrate. NOTE: Broth propagation methods may also be employed with most phage.

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## Notes

Phage is sensitive to heat, so care must be taken when adding to melted agar overlay. Plaque size is variable (from 1 to 3 mm) and hazy.

Additional information on this culture is available on the ATCC® website at [www.atcc.org](http://www.atcc.org)

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pseudoalteromonas espejiana* bacteriophage PM2 (ATCC 27025-B1)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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