Bacteriophage DO4, Infectious for *Pseudomonas aeruginosa*

Catalog No. HM-617

For research use only. Not for human use.

Contributor:
Alan Davidson, Ph.D., Professor, Department of Molecular Genetics, University of Toronto, Ontario, Canada

Manufacturer:
BEI Resources

Product Description:
Virus Classification: *Caudovirales*, *Siphoviridae*, *Unclassified Siphoviridae*
Family: *Siphoviridae*
Strain/Isolate: DO4
Host: *Pseudomonas aeruginosa*
Comments: Bacteriophage DO4 (HMP ID 9933) is a reference genome for The Human Microbiome Project (HMP). HMP is an initiative to identify and characterize human microbial flora. The complete genome of bacteriophage DO4 is currently being sequenced at the J. Craig Venter Institute.
Note: HMP material is taxonomically classified by the depositor. Quality control of these materials is only performed to demonstrate that the material distributed by BEI Resources is identical to the deposited material.

Bacteriophage DO4 is a highly selective virus that is extremely effective at lysing *Pseudomonas aeruginosa* (*P. aeruginosa*), the second most common pathogen responsible for hospital-acquired bacterial pneumonia and the first causative agent of morbidity and mortality in cystic fibrosis patients.[1,2] Bacteriophage D04 belongs to the unclassified *Siphoviridae* family of viruses, which are non-enveloped and display long, noncontractile, filamentous tails, linear dsDNA and hexagonal capsids.[3]

Material Provided:
Each vial contains approximately 0.5 mL of bacteriophage in Luria-Burtani (LB) broth supplemented with 10 mM $MgSO_4$ and 10% glycerol.

Packaging/Storage:
HM-617 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -20°C or colder immediately upon arrival. For long-term storage, the product should be stored at -80°C or colder or in the vapor phase of a liquid nitrogen freezer. Freeze-thaw cycles should be avoided.

Growth Conditions:
Host: *P. aeruginosa* (strain PA14 recommended)
Growth medium for host:
Tryptic Soy broth or equivalent
Tryptic Soy agar or equivalent
Incubation of host:
Temperature: 37°C
Atmosphere: Aerobic
Propagation of host:
Note: Host homogeneity is recommended for your intended use, please colony-purify your bacterial host prior to use.
1. Keep bacterial stock frozen until ready for use, then thaw.
2. Transfer a thawed aliquot into a single tube of broth.
3. Incubate the tube at 37°C for 24 hours.

Growth medium for bacteriophage:
LB agar supplemented with 10 mM $MgSO_4$ or equivalent
LB soft agar overlay (0.5%) supplemented with 10 mM $MgSO_4$ or equivalent
Incubation of host with bacteriophage:
Temperature: 30°C
Atmosphere: Aerobic
Propagation of bacteriophage:
1. Prior to opening the vial, an actively growing broth culture (incubate for 24 hours) of the recommended host strain should be prepared. Keep bacteriophage vial frozen until ready for use, then thaw.
2. Pre-warm plates and overlay the surface with 2.5 mL of melted 0.5% agar containing 1 to 2 drops of the host. Allow overlay to harden.
3. Prepare serial dilutions of thawed bacteriophage (if desired) and spot onto the plate. Allow to dry.
4. Incubate the plate at 30°C for 24 hours.

Note: Spotting the phage on plates makes visualizing the lysis easier. If phage is added directly to soft-agar before pouring plates, hazy or tiny plaques may be difficult to see. Resistant host bacteria may also mask plaque formation.
Cytopathic Effect: Lysis of *P. aeruginosa*; individual plaques should be countable at higher dilutions.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Bacteriophage DO4, Infectious for *Pseudomonas aeruginosa*, HM-617."

Biosafety Level: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Morello, E., et al. “Pulmonary Bacteriophage Therapy on *Pseudomonas aeruginosa* Cystic Fibrosis Strains: First Steps Towards Treatment and Prevention.” PLoS One 6 (2011): e16963. PubMed: 21347240.
2. Debarbieux, L., et al. “Bacteriophages Can Treat and Prevent *Pseudomonas aeruginosa* Lung Infections.” J. Infect. Dis. 201 (2010): 1096-1104. PubMed: 20196657.
3. Fokine, A. and M. G. Rossman. “Molecular Architecture of Tailed Double-Stranded DNA Phages.” Bacteriophage 4 (2014): e28281. PubMed: 24616838.
4. HMP ID 9933 (Bacteriophage DO4)
5. Cady, K. C., et al. "The CRISPR/Cas Adaptive Immune System of *Pseudomonas aeruginosa* Mediates Resistance to Naturally Occurring and Engineered Phages." J. Bacteriol. 194 (2012): 5728-5738. PubMed: 22885297.

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898