



# *Bdellovibrio reynosensis* Ajao et al.

TSD-288™

## Description

*Bdellovibrio reynosensis* LBG001 is a bacterial type strain that was isolated in 2019 from soil in Mexico. This type strain is an obligate predator and requires prey Gram-negative bacteria for growth. The strain grows best in HEPES complete buffer at 20-30°C at ambient temperatures.

**Strain designation:** LBG001

**Type strain:** Yes

**Type strain description:** This culture provided to the ATCC type strain depository is neither produced nor characterized by ATCC. No technical information is available on this material. Refer to depositor for technical information on this strain.

**Technical information:** ATCC Technical Services does not have technical information on type strain deposits that are not fully characterized. Additional information can be found in the depositor's publication.

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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**

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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 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****Temperature:** 20-30°C**Atmosphere:** Aerobic**Incubation:** 1 day

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**Handling Procedures**

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**Depositor-recommended growth conditions:**

Resuspend encapsulated prey bacteria cell pellet (e.g., *K. pneumoniae* ATCC 43816) to a concentration of  $1 \times 10^9$  CFU/mL (optical density at 600 nm of ~2) in sterile 25 mM HEPES complete buffer (1L HEPES supplemented with 3.33 ml of 0.6 M  $MgCl_2 \cdot 7H_2O$  and 6 ml of 0.5 M  $CaCl_2 \cdot 2H_2O$ ).

Defined or complex media can not support growth of *Bdellovibrio reynosensis*. It must be co-cultured with encapsulated prey cells. The prey cells, such as *K. pneumoniae* (ATCC 43816), are grown in rich media, centrifuged, and the cell pellet resuspended in HEPES complete buffer. Then co-culture with *B. reynosensis* (from glycerol stock, or a fresh plaque taken from a soft agar lawn of prey cells) with the prey cells in HEPES buffer. Incubate 1-3 days at 20-30°C with aeration. Monitor lysis of prey cells and appearance of small, motile *B. reynosensis* cells by Dark Field or Phase Contrast microscopy.

Depositor suggests to incubate 1-3 days at 20-30°C with aeration. The resulting lysate can be stored for up to a month at 4 °C. The lysate can be subcultured indefinitely, though it is unknown if *B. reynosensis* will mutate during repeated passaging. Consecutive subcultures can be done every 1-3 days. Alternatively the co-culture can be used to produce plaques in a DNB soft agar or soft agarose thin layer containing  $5 \times 10^9$  CFU/mL of encapsulated prey. DNB soft agar or agarose is 5 g Agar or Agarose, 0.8 g Nutrient Broth (BD Difco™ Nutrient Broth, catalog number BD 234000) in 1L H<sub>2</sub>O; autoclave; cool to 55°C before use. A plaque can then be excised with a pasteur pipet and used to seed a fresh co-culture of prey cells.

Remove residual prey cells by clarifying, then filtering a fresh lysate. Clarify by centrifugation at 3,000 RCF for 2 minutes in a microfuge tube, or for larger volumes such as 50 mL, centrifuge at 2,500 RCF for 10 minutes. Pour off and save supernatant in a new tube. Filter the supernatant through a 0.45 µm pore size filter, saving the filtrate. A 0.45 µm pore size allows passage of *B. reynosense* but not larger prey cells such as *K. pneumoniae*. For small volumes, use a 4 mm diameter syringe filter (e.g., MilliporeSigma™ Millex™ Syringe Filters with PE Housing - PVDF Membrane - 4mm Diameter, catalog number SLHVR04NL). Larger diameter syringe filters will not allow passage of *B. reynosensis*. For larger volumes use a larger diameter 0.45 µm pore size PVDF membrane filter (e.g., MilliporeSigma™ Steriflip™ Sterile Disposable Vacuum Filter Units, catalog number SE1M003M00). Add glycerol to the filtrate to 20% (v/v) and mix well prior to freezing in cryovials.

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Bdellovibrio reynosensis* Ajao et al. (ATCC TSD-288)

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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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