



Bdellovibrio exovorus

BAA-2330™

Description

Strain designation: JSS

Deposited As: *Bdellovibrio exovorus*

Type strain: No

Storage Conditions

Product format: Freeze-dried

Storage conditions: 2°C to 8°C

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.

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 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.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:

ATCC Medium 2814: YP/10 Medium

ATCC Medium 2813: DNB Medium

Temperature: 30°C**Atmosphere:** Aerobic**Incubation:** This culture must be grown in the presence of *Caulobacter crescentus* (ATCC BAA-2331)

Handling Procedures

1. Have a 24 hour host culture ready. The host, BAA-2331™, can be purchased from ATCC®. Alternatively, the host may be recovered from the *Bdellovibrio exovorus* preparation by streaking a drop on ATCC® medium #36 plate and incubating at 30°C. Once growth of the host has been obtained, a colony of the host can be picked and inoculated into ATCC® #36 broth and incubated at 30°C.

2. The *Bdellovibrio* can be propagated in a variety of ways. Open the FD vial according to the enclosed instructions.
 - A. Propagation in Broth: Inoculate 0.5 mL of a 24 hour host culture into 5 - 10 mL ATCC® medium 2814. Take 0.5 mL of this culture and add it to the vial to rehydrate the FD pellet. Add the entire content the vial back into the host culture. Incubate at 30°C with shaking
 - B. Double Layer Technique: Warm up plates of #2813, bottom agar, by placing them at 30°C for approximately one hour.
 - a. Melt tubes of top agar (2.5 – 3.0 mL agar / tube) by boiling and then placing the tubes in a 45°C water bath.
 - b. Once the top agar has cooled to 45°C, add 0.3 mL host (BAA-2331™) and 0.1 – 0.2 mL of *Bdellovibrio exovorus* (BAA-2330™), mix by rolling the tube between your hands and pour onto the bottom agar plate. Cover the bottom agar with the top agar by swirling the plate. Let the plate sit at room temperature until the top agar hardens. Incubate the inverted plates at 30°C.
3. After 24 to 48 hours, growth of the *Bdellovibrio* can be detected in the broth by a decrease in optical density. When examined by phase contrast microscope, the host cells are large, curved rods that are motile and appear singly and in pairs and clumps. The *Bdellovibrio* are small vibrio shaped cells that are very motile. As the *Bdellovibrio* continue to propagate, their densities should increase and the density of the host will decrease.
4. After 3 to 4 days of growth, plaques should appear in the top agar. Using a sterile microscope slide, one edge of the slide can be used to scrape the top agar off. The top agar can be placed in a sterile conical centrifuge tube and fresh #2814 broth can be added. Vortex, then let the particulates settle. *Bdellovibrio* can be detected under phase microscope [see step 3 above].
5. *Bdellovibrio* can be maintained for one week by storage at 4°C. *Bdellovibrio* can be stored long term by concentrating the cell by centrifugation then resuspending in maintenance medium with either 10% glycerol or 5% DMSO. Distribute the cells into cryovials, and store at a minimum of -80°C.

Notes

We have had difficulty detecting plaques using the double layer technique. Despite not detecting plaques, when the top layer has been harvested, *Bdellovibrio* have been

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detected in large quantities (almost pure) when examined by phase.

We have had great success recovering *Bdellovibrio* using the broth technique.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Bdellovibrio exovorus* (ATCC BAA-2330)

References

References and other information relating to this material are available at www.atcc.org.

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