



Clostridium ljungdahlii **Tanner et al.**

BAA-3217™

Product Sheet

Description

This product is an ATCC manufactured and accessioned progeny of 55383 cited in US Pat. No. 5,173,429.

Strain designation: PETC

Deposited As: ATCC accessioned progeny of *Clostridium ljungdahlii* strain PETC cited in US Pat. No. 5,173,429 as 55383.

Type strain: Yes

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder

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:

Anaerobe Systems Peptone Yeast Extract Broth with Glucose (PYG) (AS-822)

ATCC Medium 1754: PETC medium

ATCC Medium 2107: Modified Reinforced Clostridial

Temperature: 37°C

Atmosphere: Anaerobic; 80% H₂, 20% CO₂

Handling Procedures

1. Keep vial frozen until ready to use. This item will also grow on ATCC Medium #2107, Reinforced Clostridial Medium (BD 218081) broth and ATCC Medium

#1754, PETC broth.

2. To reduce media before inoculation, use 1.5% sodium sulfide M (0.2 mL per 10 mL). Do not use 3% Cysteine as a reducing agent.
3. The 1.5% Sodium Sulfide solution (0.2 mL per 10 mL broth) needs to be added before inoculation. If needed, exchange the gas in the Hungate tube for 80% H₂-20% CO₂. Tubes need to be pressurized to 20 psi.
4. Under anaerobic conditions, preferably in an anaerobe chamber, thaw vial and then quickly transfer entire contents into a single Hungate tube of AS-822 broth. A second tube of AS-822 broth can also be inoculated with 0.5 mL from the original broth.
5. Incubate at 35 to 37°C. Initial growth may be observed at 48 hours. Suitable growth for transfer may take up to 5 to 7 days.
6. Growth on agar plates is difficult because of the gas and pressure requirements. Better results can be achieved by producing agar slants in Hungate tubes. Agar cultures require a longer incubation period.
7. Hungate tubes need to be re-gassed and pressurized after each transfer or manipulation of the culture.
8. Growth is evident by light turbidity and an accumulation of cells at the bottom of the broth tube. A wet mount may be necessary to observe growth. This strain needs to establish the growth first in broth for 4 days before transferring. This strain does not grow well on agar. No growth is observed when using TSA+5% SB agar or Brucella agar.

ANAEROBIC CONDITIONS:

1. Hungate (or Balch) tube refers to a special type of test tube that is designed to be pressurized and is suited for anaerobic work. The Hungate test tubes can be purchased from Bellco glass (www.bellcoglass.com; stock no. 204800150).
2. Resazurin is a commonly used redox indicator that is pink when the redox potential is above -50 mv., and colorless when the redox potential is below -110 mv. i.e. highly reducing. Most strict anaerobes require this low redox potential for optimum growth.
3. To obtain a fully reduced medium, it is necessary that the medium be anoxic and that a reducing agent be added. There are several common reducing agents, however, 1.5% sodium sulfide is recommended for this strain. Add 0.2 mL of reducing agent for each 10 mL of medium.
4. Syringes can be made anaerobic by one of two methods.

- a. Displace the dead space in the syringe with a sterile oxygen-free gas.
 - b. Displace the dead space in the syringe with a reducing agent.
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Notes

Tubes need to be pressurized to 20 psi.

Recommended to use AS-822 (PYG broth) from Anaerobe Systems.

This organism will also grow on ATCC Medium #2107, Reinforced Clostridial Medium (BD 218081) broth and ATCC Medium #1754, PETC broth.

Always use freshly prepared pre-reduced media or pre-reduced media that has been previously prepared but stored under anaerobic conditions. Sodium sulfate was found to be slightly inhibitory. Do not use 3% Cysteine as a reducing agent. Resazurin in the media is a color indicator for anaerobic conditions. Observance of pink color in medium before use or during incubation shows anaerobic conditions have not been met and oxidation has occurred. Medium should be discarded.

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: *Clostridium ljungdahlii* Tanner et al. (ATCC BAA-3217)

References

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

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