Lotmaria passim

PRA-403™

Description

Strain designation: SF
Deposited As: *Crithidia mellificae*

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)
Instructions for complete medium: ATCC Medium 1034 Modified PYNFH medium (Available from ATCC as Cat. no. 327-X)
Temperature: 20-25°C
Atmosphere: Aerobic

Handling Procedures

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally**

-20°C). Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the entire contents to a T-25 flask containing 10 mL complete medium.
3. Incubate with the cap tightly sealed at 20-25°C.

Culture maintenance: Subculture at peak density (approximately every 7-10d) to a fresh T-25 flask of complete medium in the following manner:

1. Vigorously agitate the flask and aseptically transfer 0.1 – 0.2 mL to a T-25 tissue culture flask containing 10 mL complete medium.
2. Incubate with the cap tightly sealed at 20-25°C.

Reagents for cryopreservation: Cryoprotective Solution
DMSO, 1.0 mL
Fresh complete growth medium, 9.0 mL

Cryopreservation:

Harvest and Preservation

1. Harvest cells from a culture which is at or near peak density by centrifugation at 800-1000 x g for 5 min.
2. Adjust concentration of cells to between 2 x 10^7 and 2 x 10^8 cells/mL in fresh medium. If the cell concentration is too low, centrifuge at 800-1000 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. While cells are centrifuging, prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth). The DMSO solution when first prepared will warm up due to chemical heat. The solution should be allowed to return to room temperature prior to use.
4. Mix the cell preparation and the DMSO solution in equal portions. The final concentration will be 10^7 – 10^8 cells/mL and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no more than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place the ampules in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The

cooling rate in this apparatus is approximately -1°C/min.) If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C, plunge ampules into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state, place an ampule in a 35°C water bath (2-3 min). Immerse the vial just sufficiently to cover the frozen material. Do not agitate the vial.
9. Remove the vial from the water bath immediately after thawing. Aseptically transfer the entire contents of the ampule into a T-25 tissue culture flask containing 10.0 mL complete medium. Incubate with the cap tightly sealed at 20-25°C.
10. Maintain as described above.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Lotmaria passim* (ATCC PRA-403)

References

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

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