Sappinia pedata Dangeard

PRA-237™

Description

Strain designation: AUK06-2-1
Type strain: No

Storage Conditions

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 2432: wMY (weak Malt Yeast Extract)
Instructions for complete medium: ATCC Medium 2432 wMY inoculated with *Escherichia coli* (ATCC® 23437™)
Temperature: 15-20°C

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 contents to a plate of ATCC medium 2432. Distribute the material evenly over the plate using a spread bar.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 15-20°C. Amoeboid trophozoites and/or stalked fruiting bodies should be seen within 2-5 d.

Culture maintenance:

1. Prepare an ATCC medium 2432 plate with a lawn of *Escherichia coli* (ATCC® 23437™) and incubate at 37°C overnight.
2. Remove an agar block (~5 mm^2) with trophozoites from the edge of an agar plate culture and invert the block at the edge of the freshly prepared plate.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 15-20°C.
4. Repeat steps 1-3 at 7-10 d intervals.

Reagents for cryopreservation: Cryoprotective Solution

DMSO, 1.5 mL

Fresh growth medium w/o bacteria, 8.5 mL

Cryopreservation:

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest cells from a culture which is at or near peak density by adding 5 mL ATCC medium 5080 (Dryl's solution) and washing cells into suspension. Rub the surface of the plate with a spread bar to detach adhering trophozoites.
3. Adjust the concentration of cells to at least 2 x 10^4/mL in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. 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. Alternatively, place the vials 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.)

7. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, aseptically transfer the contents of the ampule to the center of a fresh plate of ATCC medium 2432. Distribute the material evenly over the plate using a spread bar.
9. Wrap the entire edge of the plate with parafilm and incubate upright at 15-20°C.
10. Follow the protocol for maintenance of culture.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Sappinia pedata* Dangeard (ATCC PRA-237)

References

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

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Revision

This information on this document was last updated on 2025-10-13

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