Entamoeba gingivalis (Gros) Brumpt

30927™

Description

Strain designation: HU-304:NIH
Deposited As: *Entamoeba gingivalis* (Gros) Brumpt
Type strain: No

Storage Conditions

Product format: Test tube
Storage conditions: See handling procedure

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 2

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 1171: TYGM-9 medium
Instructions for complete medium: ATCC Medium 1171 grown with mixed bacteria (Quality controlled lots of this medium are commercially available from ATCC as cat. no. PRA-1171)
Temperature: 35°C
Atmosphere: Microaerophilic
Culture system: Xenic
Incubation: Grown with bacteria

Handling Procedures

Handling of Live Culture

This strain is routinely shipped as a growing culture with bacteria in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 15 mL. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.** To assure viability, immediately incubate culture at a 15° horizontal slant at 35°C for at least three hours before observing the culture. There should be numerous active trophozoites attached to the tube. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, the following steps should be taken to ensure the best chance for culture survival:

1. Remove 5.5 mL of fluid and centrifuge at 500 x g for 5 minutes.
2. Inoculate two fresh tubes of ATCC medium 1171 with 0.25 mL of supernatant each from the fluid centrifuged in step 1 (preinoculated bacterized culture tubes may allow for better growth).
3. Divide the remainder of the supernatant in two equal aliquots in 16 x 125 mm screw-capped tubes and bring the volumes up to 8 mL with fresh ATCC Medium 1171.
4. Ice the parent culture 5 minutes, invert 20 times and transfer 0.5 and 1.0 mL aliquots to the test tubes containing the equal volumes of supernatant.
5. Re-feed the parent culture by centrifuging it at 200 x g for 5 min, aspirate most of the supernatant (leaving approximately 1.0-1.5 mL), and resuspend the pellet with fresh growth medium up to 8 mL.
6. Incubate all cultures (including the tubes of bacterized ATCC medium 1171) at a 15° horizontal slant at 35°C.
7. Observe the culture daily and transfer when many trophozoites are observed (i.e., early stationary phase).

Culture maintenance:

1. Ice a test tube culture at or near peak density for 10 minutes, invert 20 times and aseptically transfer a 0.1 and 0.3 mL aliquot to a fresh tube of ATCC medium 1171.
2. Screw cap on tightly and incubate on a 15° horizontal slant at 35°C. Transfer when many trophozoites are observed (i.e., early stationary phase).

Notes: In general, better growth can often be achieved by using media pre-inoculated with bacteria. Addition of penicillin G at 75 U/mL and streptomycin at 75 µg/mL to ATCC Medium 1171 may be necessary if the bacterial density in the culture is too high. Inoculate fresh tubes of media with bacteria at least one day prior to subcultivation and prior to addition of antibiotics, if used. Also, nutrients in ATCC medium 1171 may be diluted through use of an osmotically-balanced saline buffer

such as Dulbecco's PBS (ATCC 30-2200) with the medium. Mixtures in the range of 1:1 to 3:1 (medium to buffer) usually work well to control or limit bacterial growth in cultures where other methods are not sufficient.

Reagents for cryopreservation: CPMB-5 Cryoprotective Solution
DMSO, 1.0 mL
2.5 M Sucrose, 0.8 mL
L-Cysteine/Ascorbic Acid Solution, 0.2 mL
CPMB-2 Basal Solution, 6.0 mL
HIBS, 2.0 mL

CPMB-2 Basal Solution
Yeast Extract, 60.0 g
K_2HPO_4, 1.0 g
KH_2PO_4, 0.6 g
NaCl, 2.0 g
Distilled water, 1.0 L
Autoclave for 15 minutes.

L-Cysteine/Ascorbic Acid Solution
L-Cysteine-HCL, 1.0 g
Acorbic Acid, 0.1 g
Distilled water, 10.0 mL

Add 9.0 mL of distilled water to a 20 mL beaker and dissolve the first two components. While stirring, adjust the pH to 7.2 with 10N NaOH (approximately 0.7 mL). Adjust final volume to 10 mL with distilled water and filter sterilize. Solution should be used soon after preparation. Discard any unused solution.

Cryopreservation:

1. Harvest cells from several cultures which are in the late logarithmic to early stationary phase of growth. Place culture vessels on ice for 10 min.
2. Invert tubes 20 times and centrifuge at 200-300 x g for 5 min.
3. While cells are centrifuging, prepare the cryoprotective solution.
    a. Place 1.0 mL of DMSO in a 16 x 125 mm screw-capped tube and ice until solidified.
    b. Add 0.8 mL of the 2.5 M Sucrose solution, remove from ice and invert until the DMSO is liquefied. Return to ice bath.

c. Add 0.2 mL of the L-Cysteine/Ascorbic Acid solution to the DMSO solution and mix.
d. Add 6.0 mL of the CPMB-2 Basal Solution and mix.
e. Add 2.0 mL HIBS (heat-inactivated bovine serum) and mix.

4. Resuspend the cell pellets and pool to a final volume of approximately 10 mL with the supernatant. Make a determination of the cell density and adjust the concentration of the cells between 5 x 10^5/mL - 1 x 10^6/mL using fresh medium. If the cell concentration is below 5 x 10^5/mL, centrifuge the cell suspension and resuspend the pellet in a volume that will yield the desired concentration.
5. After the cell concentration is adjusted, centrifuge as in step 2.
6. Remove as much supernatant as possible and determine the volume removed.
7. Resuspend the cell pellet with a volume of the cryoprotective solution equal to the volume of the supernatant removed. Invert the tube several times to obtain a uniform cell density.
8. Dispense 0.5 mL aliquots into 1.0 - 2.0 mL plastic sterile cryules (special plastic vials for cryopreservation).
9. 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.
10. Store ampules in a liquid nitrogen refrigerator until needed.
11. One day before thawing a frozen ampule inoculate two tubes of ATCC medium 1171 with the bacterial flora only. Incubate the tube on a 15° horizontal slant at 35°C. If the specific bacterial flora associated with this culture are not available, skip this step and proceed to step 12.
12. On the following day combine 4.1 mL of the bacterized medium 1171 prepared in step 11 with 0.9 mL of HIBS (heat-inactivated bovine serum) to produce 5 mL of medium enriched with 20% serum. Invert gently several times to mix.
13. Remove the frozen ampule from liquid nitrogen and flame gently at the base of the cap. Remove the cap and aseptically add 0.5 mL of the serum-enriched medium prepared in step 12. Place in a 35°C water bath until thawed (2-3 min).
Note: Manipulations of the ampule before placing in the water bath should be done as quickly as possible to avoid warming of the contents at a suboptimal rate.
14. Transfer contents of the thawed ampule to a one-dram screw-capped vial (vial holds approximately 4.0 mL).

15. Add 2.5 mL of serum-enriched medium prepared in step 12 to the vial in dropwise fashion. Tighten the cap and incubate on a 15° horizontal slant at 35°C for 2-3 hours.
16. Ice the vial for 10 minutes, then invert gently 10 times. Centrifuge the vial at 100-200 x g for 5 min.
17. Aspirate the supernatant leaving approximately 0.5 mL. **Note:** Do not aspirate the pelleted material.
18. Replace the supernatant with 3.0 mL of the bacterized medium 1171 prepared in step 11.
19. Incubate the vial on a 15° horizontal slant at 35°C with the cap screwed on tightly. Observe the culture daily and transfer when many trophozoites are observed (i.e., early stationary phase).

Notes

This xenic culture contains the original bacterial flora present when the amoeba was first isolated. In general, addition of penicillin G at 75 U/mL and streptomycin at 75 µg/mL to ATCC Medium 1171 may be necessary if the bacterial density in the culture is too high. Inoculate fresh tubes of media with bacteria at least one day prior to subcultivation and prior to addition of antibiotics, if used.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Entamoeba gingivalis* (Gros) Brumpt (ATCC 30927)

References

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

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