



Dexiostoma campyla **(Stokes) Jankowski**

50414™

Description

Strain designation: UK33b

Deposited As: *Dexiostoma campyla* (Stokes) Jankowski

Type strain: No

Storage Conditions

Product format: Test tube

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 (ATCC Medium 1034 is available in a freeze-dried format from ATCC)

Temperature: 25°C

Culture system: Axenic

Handling Procedures

Cryopreservation:

1. Harvest cells from a culture at or near peak growth by centrifugation at 300 x g for 2 min.

2. Adjust concentration of cells to 2×10^6 /ml in fresh medium.
3. While cells are centrifuging, prepare a 13% (v/v) solution of sterile DMSO in fresh medium.
 - a) Add 1.3 ml of DMSO to an ice cold 20 x 150 mm screw-capped test tube;
 - b) Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 8.7 ml of ice cold medium;
 - c) Invert several times to dissolve the DMSO;
 - d) Allow to warm to room temperature.
4. Add a volume of the DMSO solution equal to the cell suspension volume but add in 3 equal aliquots at 2 min intervals. Thus, the final concentration of the preparation will equal 6.5% (v/v) DMSO and 10^6 cells /ml.
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 vials in a controlled rate freezing unit. Use the following cooling cycle:
From room temperature cool at
-10°C/min to the heat of fusion; from the heat of fusion to
-40°C, cool at -1°C/min. At -40°C plunge into liquid nitrogen. The cooling cycle should be initiated no less than 15 and no more than 30 minutes after the addition of DMSO to the cell preparation.
7. Store in the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state, aseptically add 0.5 ml of sterile modified PYNFH medium (ATCC Medium 1034) containing 8% (w/v) sucrose to the frozen ampule. Immediately, place in a 35°C water bath, until thawed. Immerse the

ampule just sufficient to cover the frozen material. Do not agitate the ampule.

9. Immediately after thawing, aseptically remove the contents of the ampule and gently add the material to the edge of a 20 x 100 mm petri plate containing ATCC Medium 919 (non-nutrient agar) and position on a 15 degree slant. The cell suspension will pool at the edge of the plate.

10. Continue to double the volume of the cell suspension at 10 minute intervals by adding ATCC medium 1034 containing 4% sucrose (w/v). When the volume reaches 16.0 ml place the plate in a horizontal position and incubate at 25°C.

11. On the following day, gently remove the cell suspension for the plate and transfer to a T-25 tissue culture flask. Note the volume of the suspension and add a volume of fresh medium without sucrose equal to the volume of the cell suspension. Incubate the culture at 25°C.

12. After culture has been established subculture into fresh medium without sucrose.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Dexiostoma campyla* (Stokes) Jankowski (ATCC 50414)

References

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

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