Spironucleus barkhanus Sterud et al.

50377™

Description

Spironucleus barkhanus strain NOR-1 was isolated from a muscle abscess in Atlantic salmon from Vesteraalen.

Strain designation: NOR-1
Deposited As: *Spironucleus barkhanus* Sterud et al.
Type strain: No

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

Temperature: 10-15°C
Atmosphere: Anaerobic
Culture system: Axenic

Handling Procedures

ESTABLISHING A *SPIRONUCLEUS* CULTURE FROM THE FROZEN STATE:

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 ampoules 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 1 minute. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. When completely thawed, immediately inoculate the entire contents into a single fingerling salmon.

Cryopreservation:

The following procedure should be carried out on ice:

1. Harvest parasites from infected Atlantic Salmon tissues or blood.
2. Adjust the cell concentration to 2 x 10^6 - 2 x 10^7 cells/ml using an appropriate ice-cold buffer. If the concentration of cells is too low centrifuge at 500 x g for 5 minutes. Adjust the volume of supernatant to yield the desired final cell concentration.
3. Prepare a 20% (v/v) solution of sterile DMSO in an appropriate ice-cold buffer.
4. Mix the cell preparation and the cryoprotective agent prepared in step 2 in equal portions. Thus, the final concentration will equal 10% (v/v) DMSO and 10^6 - 10^7 cells/ml. 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 longer 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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge 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. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stabile indefinitely. Those stored at temperatures above -130°C are progressively less stabile as the storage temperature is elevated. Vials should not be stored

above -55°C.

8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and immediately inoculate the entire contents into a single fingerling salmon.
10. Maintain at water temperatures between 10-15°C.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Spironucleus barkhanus* Sterud et al. (ATCC 50377)

References

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

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