Product Information Sheet for NR-51676

Borrelia afzelii, Strain Pko

Catalog No. NR-51676

For research use only. Not for human use.

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Manufacturer:
BEI Resources

Product Description:
Bacteria Classification: *Spirochaetaceae*, *Borrelia*
Species: *Borrelia afzelii*
Strain: Pko
Original Source: *Borrelia afzelii (B. afzelii)*, strain Pko was isolated in 1984 from the skin of a human with erythema migrans (Lyme borreliosis) in Germany.[1,2]
Comments: *B. afzelii*, strain Pko is reported to be multi-locus sequence type (MLST) ST-71 with a genome comprised of one circular chromosome, nine circular plasmids and seven linear plasmids.[2,3] The complete genome of *B. afzelii*, strain Pko has been sequenced (GenBank: CP000395).

B. afzelii is a motile spirochete transmitted by the hard tick *Ixodes ricinus* and a causative agent of Lyme borreliosis in Europe and Asia, where it is primarily associated with cutaneous symptoms, such as acrodermatitis chronica atrophicans.[2,4] *B. afzelii* is one of the three main Lyme borreliosis spirochetes in Eurasia and can be distinguished from *B. garinii and B. valaisiana,* by reservoir host, with rodents as the host for *B. afzelii* and birds as the host for *B. garinii and B. valaisiana.*[4,5]

Material Provided:
Each vial contains approximately 0.5 mL of bacterial culture in Revised Barbour-Stoenner-Kelly broth supplemented with 15% glycerol.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:
NR-51676 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:
Media:
Revised Barbour-Stoenner-Kelly broth or equivalent (Appendix I)
Note: Medium should be prepared fresh before each use.
Incubation:
Temperature: 32°C to 34°C
Atmosphere: Microaerophilic
Propagation:
Note: It is recommended that NR-51676 be cultured in 24-well plates until growth is established from the frozen vial.
1. Place the frozen vial in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Immerse the vial just enough to cover the frozen material. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the contents of the vial to 2 wells of a 24-well plate containing 1.5 mL fresh Revised Barbour-Stoenner-Kelly medium per well.
3. Incubate the plate at 32°C to 34°C. Do not shake culture during growth. It may take up to 21 days for the culture to establish from the frozen state.

Note: NR-51676 should be subcultured during the log phase of growth, as viability of the culture may decrease quickly.
Maintenance:
1. Monitor growth of the culture by live/dead staining every 3 to 6 days. When the culture has reached the log phase, transfer approximately 2 mL into to a T-25 tissue culture flask containing 8 mL fresh Revised Barbour-Stoenner-Kelly medium.
2. Incubate the plate at 32°C to 34°C.
3. Transfer the culture every 3 to 21 days as described in Maintenance steps 1 and 2. The transfer interval will depend on the size of the inoculum and the quality of the medium. This should be determined by performing live/dead staining every 3 to 6 days. Do not allow the culture to overgrow. Viability of the culture may be affected soon after reaching peak density.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Borrelia afzelii*, Strain Pko, NR-51676."

Biosafety Level: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Fingerle, V., Personal Communication.
2. Gallais, F., et al. "Multilocus Sequence Typing of Clinical *Borreliella afzelii* Strains: Population Structure and Differential Ability to Disseminate in Humans." Parasit. Vectors 11 (2018): 374. PubMed: 29954419.
3. Glöckner, G., et al. "Comparative Genome Analysis: Selection Pressure on the *Borrelia* vls Cassettes is Essential for Infectivity." BMC Genomics 7 (2006): 211. PubMed: 16914037.
4. van Duijvendijk, G., H. Sprong and W. Takken. "Multi-trophic Interactions Driving the Transmission Cycle of *Borrelia afzelii* Between *Ixodes ricinus* and Rodents: A Review." Parasit. Vectors 8 (2015): 643. Pubmed: 26684199.
5. Margos, G., et al. "A New *Borrelia* Species Defined by Multilocus Sequence Analysis of Housekeeping Genes." Appl. Environ. Microbiol. 75 (2019): 5410-5416. PubMed: 19542332.

Support Provided by NIAID

APPENDIX I: REVISED BSK MEDIUM (ATCC® MEDIUM: 1914)

1. Prepare the Revised BSK medium directly before each use following the recipe below by dissolving each component one at a time in distilled water:

HEPES	5.64 g
Neopeptone	4.7 g
Sodium citrate	0.7 g
Glucose	5.64 g
$NaHCO_3$	2.0 g
TC-Yeastolate	2.0 g
Sodium pyruvate	0.75 g
N-acetylglucosamine	0.37 g
Bovine serum albumin, fraction V	47.0 g
Distilled water	840 mL

2. Adjust the pH of the base medium to 7.5 using 1 N HCl or 1 N NaOH and filter-sterilize using a 0.22 µm filter.
3. Aseptically add the next two components to the base medium:

CMRL 1066 Medium, 10× (w/o Glutamine and $NaHCO_3$)	100.0 mL
Heat-inactivated rabbit serum	60.0 mL

4. Mix well and aseptically dispense into appropriate vessels. The medium may be stored in aliquots of 50 mL in freezer-safe vessels and stored frozen at -20°C until use. Once thawed, each aliquot should be kept at 2°C to 8°C and used within one month.
5. Adjust the pH of the complete medium to 7.5 to 7.6, as needed, using sterile solutions of 1 N HCl or 1 N NaOH, before use.

Note: Medium should be prepared fresh directly before each use or immediately aliquoted and frozen at -20°C until needed. Once thawed, each aliquot should be kept at 2°C to 8°C and used within one month.

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