

***Borrelia miyamotoi*, Strain HT31**

**Catalog No. NR-51675**

**For research use only. Not for human use.**

**Contributor:**

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**Manufacturer:**

BEI Resources

**Product Description:**

Bacteria Classification: Spirochaetaceae, *Borrelia*

Species: *Borrelia miyamotoi*

Strain: HT31

Original Source: *Borrelia miyamotoi* (*B. miyamotoi*), strain HT31 was isolated from the abdomen of an unfed female *Ixodes persulcatus* tick collected from vegetation between 1990 and 1992 in Shiretoko, Hokkaido, Japan.<sup>1,2</sup>

Comments: *B. miyamotoi*, strain HT31 is the type strain of the species and is described within the Asian/Siberian genotypic clade.<sup>3</sup>

*B. miyamotoi* is a motile spirochete and the causative agent of tick-borne relapsing fever (TBRF), and an emerging human pathogen in the United States, Europe and Asia.<sup>4</sup> Unlike other spirochetes in the relapsing-fever group, which are transmitted by soft-bodied ticks, *B. miyamotoi* is transmitted by hard-bodied ticks in the *Ixodes* genus, the same vectors of Lyme disease (*B. burgdorferi*), with which it co-circulates.<sup>3,4</sup> *B. miyamotoi* is clustered into three genotypic clades based on geographical and vector distribution, Asian/Siberian (*Ixodes persulcatus* (*I. persulcatus*)/*I. pavlovskyi*), American (*I. scapularis* or *I. pacificus*), European (*I. ricinus*), with a potential fourth clade, Japanese (*I. ovatus*) recently described.<sup>3</sup>

**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-51675 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: Aerobic with 5% CO<sub>2</sub>

Propagation:

Note: It is recommended that NR-51675 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-51675 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 miyamotoi*, Strain HT31, NR-51675."

**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/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

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**References:**

1. Fingerle, V., Personal Communication.
2. Fukunaga, M., et al. "Genetic and Phenotypic Analysis of *Borrelia miyamotoi* sp. nov., Isolated from the Ixodid Tick *Ixodes persulcatus*, the Vector for Lyme Disease in Japan." *Int. J. Syst. Bacteriol.* 45 (1995): 804-810. PubMed: 7547303.
3. Cutler, S., et al. "A New *Borrelia* on the Block: *Borrelia miyamotoi* – A Human Health Risk?" *Euro. Surveill.* 24 (2019): 1800170. PubMed: 31064634.
4. Krause, B. J., et al. "*Borrelia miyamotoi* Infection in Nature and in Humans." *Clin. Microbiol. Infect.* 21 (2015): 631-639. PubMed: 25700888.

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**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 <sub>3</sub>	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 <sub>3</sub> )	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.