

***Treponema pallidum* subsp. *pallidum*,
Strain Nichols (Houston) (*in vitro*)**

Catalog No. NR-59701

For research use only. Not for use in humans.

Contributor and Manufacturer:

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Product Description:

Bacteria Classification: *Treponemataceae*, *Treponema*

Species: *Treponema pallidum* subsp. *pallidum*

Strain: Nichols (Houston)

Original Source: *Treponema pallidum* (*T. pallidum*) subsp. *pallidum*, strain Nichols (Houston) was deposited to BEI Resources as a clone of strain Nichols, adapted to *in vitro* culture in *Sylvilagus floridanus* (cottontail rabbit) epithelial cells (ATCC® CCL-68™).^{1,2,3,4} Strain Nichols (Houston) is a continually propagated laboratory clone of strain Nichols established in 1982.^{1,2} The parental strain Nichols was originally isolated in 1912 from the cerebrospinal fluid of a human subject with recurrent neurosyphilis following treatment with neosalvarsan in Washington, D.C., USA.^{5,6}

Comments: Strain Nichols (Houston) is suitable for propagation both *in vitro* and *in vivo*.^{1,7} The complete genome of *T. pallidum* subsp. *pallidum*, strain Nichols has been sequenced (GenBank: [AE000520](#)).

T. pallidum subsp. *pallidum* is a pathogenic, obligate spirochete and the causative agent of syphilis, an invasive and persistent sexually transmitted disease with increasing incidence in the United States and worldwide.^{2,3,4} Long-term propagation has been limited to rabbits since it was first identified in 1905.^{5,6,8} The recent optimization of an *in vitro* method in *Sylvilagus floridanus* epithelial cells with a specialized growth medium has allowed for continuous propagation while maintaining the characteristic ultrastructure and full infectivity *in vivo*.^{4,8}

Material Provided:

Each vial of NR-59701 contains approximately 1.25 mL of bacterial culture in phosphate buffer saline containing 50% heat-inactivated rabbit serum and 15% glycerol. The concentration is reported on the Certificate of Analysis.

Packaging/Storage:

NR-59701 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:

T. pallidum Culture Medium 2 (TpCM-2)⁴ (Appendix I)

Host:

Sylvilagus floridanus (cottontail rabbit) epithelial cells (Sf1Ep; ATCC® CCL-68™) or equivalent

Incubation:

Temperature: 34°C

Atmosphere: Microaerophilic (1.5% O₂; 5% CO₂; 93.5% N₂)

Please refer to Appendix II for preparation of host cell monolayer, Appendix III for propagation of NR-59701, Appendix IV for maintenance of NR-59701 cultures and Appendix V for cryopreservation instructions.

Note: Complete protocols and additional information and instructions are provided in: Edmondson, D. G. and S. J. Norris. "*In Vitro* Cultivation of the Syphilis Spirochete *Treponema pallidum*." *Curr. Protoc.* 1 (2021): e44. PubMed: 33599121. Please refer to this reference before starting work with NR-59701.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Treponema pallidum* subsp. *pallidum*, Strain Nichols (Houston) (*in vitro*), NR-59701."

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

Disclaimers:

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References:

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- Edmondson, D. G., et al. "Clonal Isolates of *Treponema pallidum* subsp. *pallidum* Nichols Provide Evidence for the Occurrence of Microevolution During Experimental Rabbit Infection and *In Vitro* Culture." *PLoS One* 18 (2023): e0281187. PubMed: 36917571.
- Edmondson, D. G., et al. "Parameters Affecting Continuous *In Vitro* Culture of *Treponema pallidum* Strains." *mBio* 12 (2021): e03536-20. PubMed: 33622721.
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- Nichols, H. J. "Observations on a Strain of *Spirochaeta pallida* Isolated from the Nervous System." *J. Exp. Med.* 19 (1914): 362-371. PubMed: 19867775.
- Nichols, H. J. and W. H. Hough. "Demonstration of *Spirochaeta pallida* in the Cerebrospinal Fluid From a Patient with Nervous Relapse Following the Use of Salvarsan." *JAMA* 60 (1913): 108-110.
- Lukehart, S. A. and C. M. Marra. "Isolation and Laboratory Maintenance of *Treponema pallidum*." *Curr. Protoc. Microbiol.* 12 (2007): 12A.1. PubMed: 18770607.
- Edmondson, D. G., B. Hu and S. J. Norris. "Long-Term *In Vitro* Culture of the Syphilis Spirochete *Treponema pallidum* subsp. *pallidum*." *mBio* 9 (2018): e01153-18. PubMed: 29946052.
- Norris, S. J. and D. G. Edmondson. "Compositions and Methods For Long-Term *In Vitro* Culture of the Syphilis Spirochete." [U.S. Patent No. 11,130,937 B2](#), 2021.

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Note: For complete instructions, protocols and additional information, please refer to: Edmondson, D. G. and S. J. Norris. "*In Vitro* Cultivation of the Syphilis Spirochete *Treponema pallidum*." *Curr. Protoc.* 1 (2021): e44. PubMed: 33599121.

APPENDIX I: PREPARATION OF *T. PALLIDUM* CULTURE MEDIUM 2 (TpCM-2)

- Prepare fresh TpCM-2 medium following the recipe below one day prior to culture initiation. Filter sterilize using a 0.2 µm filter.

Note: TpCM-2 medium may be stored at -20°C for up to 6 months prior to use in a non-"frost-free" freezer (no defrosting cycle) with no loss in efficacy. Prior to use, loosen the cap of the tube containing the frozen medium and thaw overnight in a microaerophilic (1.5% O₂; 5% CO₂; 93.5% N₂) atmosphere.

Components	Volume for 50 mL	Final Concentration
CMRL1066 Medium without phenol red or L-glutamine	37 mL	0.8×
100 mM Sodium Pyruvate Solution	364 µL	0.73 mM
0.1% (w/v) Resazurin	50 µL	0.001%
1M MOPS, pH 7.5	1 mL	20 mM
7.5% (w/v) Sodium Bicarbonate Solution	1.08 mL	19.2 mM
200 mM L-Glutamine Solution	500 µL	2 mM
100× D-glucose (15% solution in water)	500 µL	to 17.6 mM
10 g/dL D-mannitol (10% in water)	80 µL	0.88 mM
5 g/dL L-histidine (5% in water)	80 µL	0.52 mM
DL-Dithiothreitol	4 mg	0.52 mM
Heat-Inactivated Fetal Bovine Serum	10 mL	20%

- Pre-gas fresh TpCM-2 by placing the container (with a loosened lid) in a vented Brewer jar. Exchange the atmosphere in the jar five times. Connect the Brewer jar to a vacuum source and gauge with vacuum tubing and connectors (see Figure 1 in

- Edmondson, D. G. and S. J. Norris. "In Vitro Cultivation of the Syphilis Spirochete *Treponema pallidum*." *Curr. Protoc.* 1 (2021): e44. PubMed: 33599121.). Draw a vacuum (12 in. of Hg to 18 in. of Hg) in the jar using the vacuum source. Refill the jar with 5% CO₂ and 95% N₂. Repeat vacuum-and-refilling procedure four additional times using 1.5% O₂; 5% CO₂; 93.5% N₂ for the final refill.
- Quickly transfer the pre-gassed TpCM-2 to a humidified incubator maintained at 34°C with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂) and continue pre-equilibration overnight. Leave the lid of the medium container loose to facilitate pre-equilibration. An incubator tank switch may be used, if preferred. Alternatively, after gas exchange in the Brewer jar, seal the gas inlet of the jar with a hose clamp and transfer the entire jar to a 34°C incubator.

APPENDIX II: PREPARATION OF THE HOST CELL MONOLAYER

- Prepare the Sf1Ep medium following the recipe below. Filter sterilize using a 0.2 µm filter.

Note: Sf1Ep medium may be stored at 4°C for up to 3 months.

Components	Volume
Minimal Essential Medium Eagle (MEM) with Earle's salts and L-glutamine	500 mL
MEM Non-Essential Amino Acids Solution	5 mL
100 mM Sodium Pyruvate Solution	5 mL
200 mM L-Glutamine Solution	5 mL
Heat-Inactivated Fetal Bovine Serum	50 mL

- One day prior to culture initiation or passage, seed *Sylvilagus floridanus* epithelial cells (Sf1Ep; ATCC® CCL-68™) into 6-well tissue culture plates with low-evaporation lids at a seeding density of 0.5×10^5 to 1×10^5 cells/well in Sf1Ep medium.
- Incubate the inoculated plates overnight at 37°C in an aerobic atmosphere with 5% CO₂ until the cells form a 10% confluent, adherent monolayer.

APPENDIX III: INITIATION OF IN VITRO CULTURE OF NR-59701

- Three to four hours prior to the start of an experiment or passage, obtain the 6-well plates containing the Sf1Ep monolayers. Remove Sf1Ep medium from the 6-well plates by aspiration.
- Rinse each well with 1 mL pre-equilibrated TpCM-2 (Appendix I) to remove traces of Sf1Ep medium. Aspirate and discard the TpCM-2 rinse.
- Add 4 mL pre-equilibrated TpCM-2 (Appendix I) to each well.
- Place 6-well plates in a Brewer jar. Replace the air in the Brewer jar with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂) following the instructions in Appendix I.
- Quickly transfer plates to an incubator maintained at 34°C with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂) and allow to equilibrate for at least 3 to 4 hours.
- Thaw a frozen vial of NR-59701 in a 37°C water bath for 2 to 3 minutes, until thawed. Immerse the vial just enough to cover the frozen material. Do not agitate the vial. NR-59701 may exhibit sluggish motility following thawing.
- Dilute the vial contents such that the inoculum contains 2×10^6 to 5×10^6 motile *T. pallidum*. The inoculum volume is typically 200 µL to 750 µL, although lower or higher volumes may be used.
- Remove Sf1Ep cultures from the microaerophilic incubator (step 5).
- Inoculate each well of the 6-well plate containing the Sf1Ep host cell monolayer with prepared NR-59701 at the appropriate inoculation volume.
- Place the inoculated 6-well plates in the Brewer jar and replace air in the jar with the microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂) following the instructions in Appendix I.
- Quickly transfer plates to the incubator maintained at 34°C with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂). Be sure that the base of the culture vessel is well supported during transport to avoid accidental spillage.
- Incubate cultures for 7 days at 34°C with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂).

Note: *T. pallidum* has a very slow doubling time of 32 hours to 40 hours. Seven days provides for 4 to 5 generations and is a convenient interval between subcultures. Culture time can be extended a few days by feeding *T. pallidum* cultures every 3 to 5 days by removing half the TpCM-2 and replacing it with fresh medium. Despite this procedure, the Sf1Ep cultures may overgrow and fail to support *T. pallidum* growth after 10 to 14 days of culture.

APPENDIX IV: MAINTENANCE AND HARVEST OF *IN VITRO* CULTURE OF NR-59701

1. One day prior to harvest or passage, prepare fresh TpCM-2 medium (Appendix I) and Sf1Ep cultures (Appendix II).
2. On the day of passage, change the medium of the Sf1Ep cultures and pre-equilibrate following the instructions in Appendix II.
3. Immediately prior to harvest, remove *T. pallidum* cultures from the microaerophilic incubator and examine with an inverted microscope to assess confluency and overall appearance of the host cell monolayer.
4. Remove the TpCM-2 growth medium from each well by aspiration and reserve it in separate sterile 15 mL conical centrifuge tubes. Measure the volume of medium to ensure that there has not been significant evaporation.
5. Rinse each well with 0.35 mL trypsin-EDTA and transfer the rinse to the tube containing the reserved medium.
6. Add an additional 0.35 mL trypsin-EDTA to each well.
7. Incubate plates at 37°C in an aerobic atmosphere with 5% CO₂ for 5 minutes.
8. View plates with the inverted microscope to ensure that the Sf1Ep cells are rounded or floating. Rap the edge of each plate to dislodge the Sf1Ep cells from the plate and the *T. pallidum* from the cells.
9. Complete trypsinization is important to maximize *T. pallidum* recovery. Typically, ~ 50% of the *T. pallidum* are attached to the Sf1Ep monolayer in a healthy culture, and these are released by trypsinization. Hold the 6-well plate flat on the stage of the inverted microscope and rap the side of the plate with a polypropylene rack for microcentrifuge tubes to dislodge the cells from the plate. If the cells do not dislodge, add additional trypsin-EDTA and incubate at 37°C for 5 minutes more.
10. When trypsinization is complete and all the Sf1Ep cells are detached, add reserved medium (see step 5) back to the corresponding well to stop the trypsinization.

Note: When the use of trypsin might be deleterious to an experiment, please refer to the alternative protocol provided in: Edmondson, D. G. and S. J. Norris. "In Vitro Cultivation of the Syphilis Spirochete *Treponema pallidum*." Curr. Protoc. 1 (2021): e44. PubMed: 33599121.

11. Rinse well with the reserved medium and return the combined trypsin and medium to the sterile 15 mL conical centrifuge tube.

Optional: Centrifuge for 5 minutes at 125 × g to pellet Sf1Ep cells. This step reduces variability due to Sf1Ep cell "carryover."

 - a) Use 125 µL to 750 µL (typically 250 µL) of the trypsinized, optionally centrifuged culture to inoculate new 6-well Sf1Ep cultures prepared in steps 1 and 2. Three replicate cultures per condition are recommended. Adjust the volume to yield an inoculum of 2 × 10⁶ to 6 × 10⁶ *T. pallidum*.
 - b) Gas the inoculated cultures in the Brewer jar following the instructions in Appendix I and return new cultures to the incubator maintained at 34°C with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂).
12. Quantify harvested NR-59701 by darkfield microscopy. Please refer to Support Protocol 3 provided in: Edmondson, D. G. and S. J. Norris. "In Vitro Cultivation of the Syphilis Spirochete *Treponema pallidum*." Curr. Protoc. 1 (2021): e44. PubMed: 33599121.

APPENDIX V: CRYOPRESERVATION

1. Transfer 1 mL of the trypsinized culture from Appendix IV to a cryovial.
2. Add 250 µL of sterile 50% glycerol to yield a final glycerol concentration of 10%.
3. Mix gently by pipetting or inversion and place the vial at -80°C.