Product Information Sheet for NR-58893

Trichomonas vaginalis, Strain NYCC30

Catalog No. NR-58893

For research use only. Not for use in humans.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Trichomonadidae*, *Trichomonas*
Species: *Trichomonas vaginalis*
Strain: NYCC30
Original Source: *Trichomonas vaginalis (T. vaginalis)*, strain NYCC30 was isolated in 2008 from a human with symptomatic trichomoniasis in New York, New York, USA.[1,2]
Comment: Strain NYCC30 was deposited to BEI Resources as a genotype type 2 strain sensitive to metronidazole and negative for the *T. vaginalis* virus (TVV).[1,2,3]

T. vaginalis is the most common non-viral, sexually transmitted parasite in humans and causative agent of trichomoniasis.[2,3,4,5] Metronidazole is the drug of choice for treatment, however resistance to this antibiotic has been observed in some clinical cases.[2,3] The unique population structure of this genetically diverse parasite consists of two genotypes, 1 and 2, present in equal proportions world-wide.[3] These genotypes differ in the rate at which they harbor the *T. vaginalis* virus (TVV), a non-segmented dsRNA virus of the family *Totiviridae* involved in *T. vaginalis* virulence and disease pathogenesis, and in their sensitivity to metronidazole.[3,4,5] Infection of *T. vaginalis* with TVV is associated primarily with genotype type 1 strains and increased susceptibility to metronidazole.[2,4]

Material Provided:
Each vial of NR-58893 contains approximately 0.5 mL of cells in cryopreservative [5% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-58893 was packaged aseptically in cryovials and is provided frozen on dry ice. The product should be stored at -130°C or colder, preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To ensure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:
Modified Trypticase – Yeast – Maltose (TYM) Basal medium supplemented with 10% heat-inactivated horse serum (HIHS) and 0.71% iron (Appendix II) or equivalent
Incubation:
Temperature: 35°C
Atmosphere: Microaerophilic
Propagation:
1. To establish a culture from the frozen state, place a vial in a 35°C to 37°C water bath. Thawing time is approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Transfer the vial contents to a 16 × 125 mm screw-capped borosilicate glass test tube containing 13 mL of growth medium.
3. Screw the cap on tightly and incubate at a 15° horizontal slant at 25°C. Observe the culture daily and subculture when peak density is observed.

Maintenance:
1. When the culture is at or near peak density, ice the culture for 10 minutes and gently invert 20 times.
2. Add 12 mL of freshly prepared growth media to two sterile tubes.
3. Transfer every 2 to 3 days, as needed. Note that the transfer interval should be determined empirically as it is dependent on the quantity of the inoculum.
4. Aseptically transfer a 100 µL to 250 µL aliquot of *T. vaginalis*, strain NYCC30 culture to the tubes prepared in step 2.
5. Screw the cap on tightly and incubate at a 15° horizontal slant at 25°C. Observe the culture daily and subculture when peak density is observed.

Please refer to Appendix I for cryopreservation instructions.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through the BEI Resources, NIAID, NIH: *Trichomonas vaginalis,* Strain NYCC30, NR-58893."

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:

1. Carlton, J., Personal Communication.
2. Bradic, M., et al. "Genetic Indicators of Drug Resistance in the Highly Repetitive Genome of *Trichomonas vaginalis*." Genome Biol. Evol. 9 (2017): 1658-1672. PubMed: 28633446.
3. Conrad, M. D., et al. "Extensive Genetic Diversity, Unique Population Structure and Evidence of Genetic Exchange in the Sexually Transmitted Parasite *Trichomonas vaginalis*." PLoS Negl. Trop. Dis. 6 (2012): e1573. PubMed: 22479659.
4. Graves, K. J., et al. "*Trichomonas vaginalis*: A Review of the Literature." Int. J. STD. AIDS 30 (2019): 496-504. PubMed: 30626281.
5. Edwards, T., et al. "*Trichomonas vaginalis*: Clinical Relevance, Pathogenicity and Diagnosis." Crit. Rev. Microbiol. 42 (2016): 406-417. PubMed: 25383648.

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APPENDIX I: CRYOPRESERVATION

1. Harvest cells from several *Trichomonas* cultures that are in peak density of growth and place on ice for 10 minutes.
2. Gently invert tubes several times and centrifuge at 800 × g for 5 minutes.
3. While cells are centrifuging, prepare the Cryoprotective Solution [10% (v/v) solution of sterile DMSO in fresh medium]:
    a. Add 1 mL of DMSO to a 20 × 150 mm screw-capped test tube and place on ice until solidified (approximately 5 minutes).
    b. Add 9 mL of ice cold incomplete modified TYM medium and invert until the DMSO is liquefied.
    c. Allow the solution to warm to room temperature.
4. Resuspend the cell pellets and pool to a final volume of approximately 10 mL with fresh complete Modified TYM Basal medium supplemented with 10% HIHS and 25 mM iron.
5. Determine the cell density using a hemocytometer and adjust the concentration to between 2×10^6 to 2×10^7 cells/mL with fresh complete Modified TYM Basal medium supplemented with 10% HIHS and 25 mM iron.
   Note: If the concentration of cells is too low, centrifuge at 800 × g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
6. Mix equal volumes of cell suspension and the Cryoprotective Solution prepared above to yield a final concentration of 1×10^6 to 1×10^7 cells/mL in 5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300™) may be added to a final concentration of 50 IU/mL to 100 IU/mL penicillin and 50 µg/mL to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic cryovials.
8. Place the vials in a controlled rate freezing unit. From room temperature, cool at -10°C per minute until the liquid begins to freeze; from this point until -40°C is reached, cool at -1°C per minute. At -40°C plunge the vials into liquid nitrogen. The cooling cycle should be initiated 15 to 30 minutes after the addition of DMSO to the cell preparation.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: MODIFIED TRYPTICASE – YEAST – MALTOSE (TYM) BASAL MEDIUM

1. Prepare the Iron Solution following the recipe below and filter sterilize using a 0.2 µm filter. Store at 2°C to 8°C and discard when the solution changes from pale pink to a dark color.

Iron Solution	
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.5 g
$C_7H_6O_6S$	0.05 g
Distilled, deionized water	50 mL

2. Prepare the incomplete Modified TYM Basal medium by adding all of the dry components listed to the distilled water:

Incomplete Modified TYM Basal medium	
Trypticase	20 g
Yeast Extract	10 g
Maltose	5 g
L-Cystine • HCl	1 g
L-Ascorbic acid	0.2 g
K_2HPO_4	0.8 g
KH_2PO_4	0.8 g
Distilled water	900 mL

3. Adjust pH to 6.8 with 1 N NaOH.
4. Dispense 9 mL volumes into screw-capped tubes and autoclave at 121°C for 15 minutes and allow to cool.

   Note: Leave caps loose while autoclaving. Tighten the caps immediately upon removing the tubes from the autoclave. This medium can be stored for about one week under refrigeration. Fresh medium is required for growth of cultures. Keep tubes tightly capped at all times.

5. Aseptically prepare the Complete Modified TYM Basal medium following the recipe below:

Complete Modified TYM Basal medium	
Incomplete Modified TYM Basal medium	45 mL
Heat-Inactivated Horse Serum	5 mL
Iron Solution	0.35 mL

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