Mycobacterium tuberculosis, Strain CDC1551, Transposon Mutant 199 (MT3000, Rv2931)

Catalog No. NR-14740

For research use only. Not for human use.

Contributor:
NIH - TB Vaccine Testing and Research Materials Contract

Manufacturer:
BEI Resources

Product Description:
Bacteria Classification: *Mycobacteriaceae, Mycobacterium*
Species: *Mycobacterium tuberculosis*
Strain: CDC1551 (also referred to as CSU93 or Oshkosh)
Transposon Mutant: 199 (MT3000, Rv2931)[1-3]
TN: CQ0699
ID: TnMT3000_55
Original Source: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain CDC1551 is a clinical isolate that exhibited high levels of infectivity and virulence during a tuberculosis outbreak that occurred in rural Kentucky and Tennessee from 1994 to 1996.[4]
Comments: In 2002, TARGET (Tuberculosis Animal Research and Gene Evaluation Taskforce) was formed to enable the modeling of human tuberculosis in multiple animal species using defined protocols and testing defined mutants of *M. tuberculosis*. In addition to animal modeling activities, a library of intragenic transposon mutants has been created and characterized.[5] *M. tuberculosis,* transposon mutant 199 was created by disruption of a polyketide synthase (MT3000, Rv2931) of the wild-type strain CDC1551.

M. tuberculosis is a Gram-positive, rod-shaped aerobic bacterium. It is the causative agent of tuberculosis and is responsible for more morbidity in humans than any other bacterial disease.[6]

Material Provided:
Each tube contains a Lowenstein-Jensen (LJ) agar slant that was inoculated with 0.1 mL of bacterial culture and incubated 2 to 6 weeks at 37°C.

Packaging/Storage:
NR-14740 was packaged aseptically in screw-capped glass test tubes. This product is provided at room temperature and should be stored at 2°C to 8°C upon arrival. Do not freeze.

Growth Conditions:
Media:
Lowenstein-Jensen Agar slants (VWR catalog no. 29447-808), Middlebrook 7H10 Agar (BD 295964) with OADC enrichment (BD 212240) or Middlebrook 7H11 Agar (VWR catalog no. 29447-102) with OADC enrichment
Incubation:
Temperature: 37°C
Atmosphere: Aerobic
Propagation:
Please refer to the attached document, SOP: TN002 provided by the TB Vaccine Testing and Research Materials Contract.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Mycobacterium tuberculosis*, Strain CDC1551, Transposon Mutant 199 (MT3000, Rv2931), NR-14740."

Biosafety Level: 3
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. TARGET: Rv2931
2. JCVI: MT3000
3. TubercuList: Gene Rv2931
4. Valway, S. E., et al. "An Outbreak Involving Extensive Transmission of a Virulent Strain of *Mycobacterium tuberculosis*." N. Engl. J. Med. 338 (1998): 633-639. PubMed: 9486991.
5. Lamichhane, G., et al. "A Postgenomic Method for Predicting Essential Genes at Subsaturation Levels of Mutagenesis: Application to *Mycobacterium tuberculosis*." Proc. Natl. Acad. Sci. U. S. A. 100 (2003): 7213-7218. PubMed: 12775759.
6. Ducati, R. G., et al. "The Resumption of Consumption – A Review on Tuberculosis." Mem. Inst. Oswaldo Cruz 101 (2006): 697-714. PubMed: 17160276.
7. Cole, S. T., et al. "Deciphering the Biology of *Mycobacterium tuberculosis* from the Complete Genome Sequence." Nature 393 (1998): 537-544. PubMed: 9634230. Erratum in: Nature 396 (1998): 190-198.
8. de la Paz Santangelo, M., et al. "Mce3R, a TetR-Type Transcriptional Repressor, Controls the Expression of a Regulon Involved in Lipid Metabolism in *Mycobacterium tuberculosis*." Microbiology 155 (2009): 2245-2255. PubMed: 19389781.

Support Provided by NIAID

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SOP: TN002

Obtaining Cells from Inoculated Transposon Mutant LJ Slants

Materials and reagents:

1. *M. tuberculosis,* transposon mutant LJ slant
2. Biosafety cabinet
3. Sterile aerosol resistant pipet tips, 200 µL
4. Pipetman, 200 µL
5. Cell scraper, sterile
6. 7H9 media (note 3)
7. 7H11 + OADC agar plate, 100 x 15 mm (VWR catalog no. 29447-102)
8. Cold room or 4°C refrigerator

Protocol:

1. Remove LJ slant from container within biosafety cabinet (note 1).
2. Add 200 µL of 7H9 media to LJ slant.
3. Use cell scraper to lightly scrape the cells on the LJ slant into the 7H9 media.
4. Pipet 100 µL of the media, which now contains cell growth, onto a small 7H11 + OADC plate (note 2).
5. Streak the bacteria to grow as a lawn.
6. Place inoculated plates in a Ziploc bag, seal, and place in warm room (note 4).
7. Once cells have grown, move plates into biosafety cabinet (note 5).
8. Inside the biosafety cabinet, use a sterile cell scraper and aseptically scrape the cells into GAS media or liquid media of choice.

Notes:

1. The LJ slants must be removed from packaging only within a BSL3 facility and opened only within a BSL3 biosafety cabinet.
2. Use an aerosol resistant tip and pipetman to transfer cells from the liquid culture to the 7H11 plate. If preparing your own agar plates, follow the instructions on the bottle of 7H11 powder (Fisher Scientific catalog # DF0838-17-9).
3. Follow the instructions on the bottle of 7H9 powder (VWR catalog # 90003-876).
4. LJ slants can be kept in a cold room or 4°C refrigerator for future use.
5. Depending upon the strain, a lawn could take 2 to 4 weeks to form.

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