Product Information Sheet for NR-59698

Mycobacterium smegmatis, Strain GMC_MSM2

Catalog No. NR-59698

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Mycobacteriaceae*, *Mycobacterium*

Species: *Mycobacterium smegmatis* (also referred to as *Mycolicibacterium smegmatis*)[1,2,3,4]

Strain: GMC_MSM2

Original Source: *Mycobacterium smegmatis (M. smegmatis)*, strain GMC_MSM2 is a genetically modified strain generated by the insertion of a 3×FLAG sequence into the chromosomal DNA of *M. smegmatis*, strain mc(2)155 after the α-subunit Ser 518 residue of the ATP synthase via the oligonucleotide-mediated recombineering followed by Bxb1 integrase targeting (ORBIT) method.[5,6,7] Strain GMC_MSM2 contains plasmids pKM444 (Addgene 108319), expressing Che9c phage RecT annealase and Bxb1 phage integrase, and the hygromycin-resistant plasmid pKM491 (Addgene 109282), in which the FLAG-His sequence was replaced with the sequence encoding a 3×FLAG tag. The parent strain mc(2)155 is a fast-growing, nonpathogenic, plasmid-cured mutant used extensively as a model cell line since its isolation in 1990.[8,9]

Comments: Strain GMC_MSM2 was deposited to BEI Resources for use in a compound screening assay to detect and characterize inhibitors of mycobacterial phosphorylation.[7] The assay utilizes acidification of prepared inverted membrane vesicles (IMVs), either through the activity of the electron transport chain or ATP synthase, from two *M. smegmatis* strains, QcrB-3×FLAG (BEI Resources NR-59699) and GMC_MSMS2. Strain GMC_MSMS2 has truncated ATP synthase resulting in the activation of ATP hydrolysis activity of the ATP synthase. Strain QcrB-3×FLAG has an intact ATP synthase not capable of ATP hydrolysis.[5,7] Inhibitors of ATP synthase block ATP-driven acidification of IMVs of strain GMS_MSM2. Inhibitors of the mycobacterial $CIII_2CIV_2$ supercomplex block succinate-driven acidification of vesicles of QcrB-3×FLAG. Non-specific membrane active uncouplers block these processes in vesicles from both strains.

Note: It is recommended to order NR-59698 and NR-59699 together. For more information and complete assay details, please refer to: Harden, S. A., et al. "A Simple Assay for Inhibitors of Mycobacterial Oxidative Phosphorylation." J. Biol. Chem. 300 (2024): 105483. PubMed: 37992805.

M. smegmatis is a fast-growing, nonpathogenic, saprophytic bacterium first described in 1884. It is widely used as a model organism for mycobacterium research due to the large number of orthologous proteins shared with other species of mycobacteria, including species within the *M. tuberculosis* species complex.[8]

Reclassification of *M. smegmatis* to the novel genera *Mycolicibacterium* has been proposed following a comprehensive phylogenomic analysis of the genus *Mycobacterium*, and is currently under debate.[1,2,3,4,8]

Material Provided:

Each vial contains approximately 0.5 mL of bacterial culture in Middlebrook 7H9 broth with ADC enrichment, 0.5% Tween and 50 µg/mL hygromycin supplemented with 10% glycerol.

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

Packaging/Storage:

NR-59698 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:

Middlebrook 7H9 broth with ADC enrichment supplemented with 0.5% Tween and 50 µg/mL hygromycin or equivalent

Middlebrook 7H10 agar with OADC enrichment supplemented with 0.5% Tween and 50 µg/mL hygromycin or equivalent

Incubation:

Temperature: 37°C

Atmosphere: Aerobic

Propagation:

1. Keep vial frozen until ready for use; then thaw.
2. Transfer the entire thawed aliquot into a single tube of broth.
3. Use several drops of the suspension to inoculate an agar slant and/or plate.
4. Incubate the tube, slant and/or plate at 37°C for 2 to 3 days.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Mycobacterium smegmatis*, Strain GMC_MSM2, NR-59698."

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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2. Nouioui, I., et al. "Genome-Based Taxonomic Classification of the Phylum *Actinobacteria*." Front. Microbiol. 9 (2018): 2007. PubMed: 30186281.
3. Gupta, R. S. "Commentary: Genome-Based Taxonomic Classification of the Phylum *Actinobacteria*." 10 (2019): 206. PubMed: 30853945.
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5. Rubinstein, J., Personal Communication.
6. Guo, H.., et al. "Structure of Mycobacterial ATP Synthase Bound to the Tuberculosis Drug Bedaquiline." Nature 589 (2021): 143-147. PubMed: 33299175.
7. Harden, S. A., et al. "A Simple Assay for Inhibitors of Mycobacterial Oxidative Phosphorylation." J. Biol. Chem. 300 (2024): 105483. PubMed: 37992805.
8. Sparks, I. L., et al. "*Mycobacterium smegmatis*: The Vanguard of Mycobacterial Research." J. Bacteriol. 205 (2023): e0033722. PubMed: 36598232.
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10. Murphy, K. C., et al. "ORBIT: A New Paradigm for Genetic Engineering of Mycobacterial Chromosomes." mBio 9 (2018): e01467-18. PubMed: 30538179.

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