

***Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plates 1-8**

Catalog No. NR-19438

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Contributor:

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

Manufacturer:

BEI Resources

Product Description:

NR-19438 consists of Plates 1-8 (BEI Resources NR-19783 through NR-19789 and NR-20324) of the *Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clone Set. Detailed information is available on the Product Information Sheet and Certificate of Analysis for each individual plate.

The *Mycobacterium tuberculosis* (*M. tuberculosis*) Knockout Gateway® clone set consists of 8 plates which contain 641 sequence validated knockout clones from *M. tuberculosis*, strain CDC1551. Each open reading frame was constructed with a hygromycin selectable gene replacement marker in vector pDEST-YUB, a Gateway® compatible adaptation of the cosmid cloning vector pYUB854¹ and cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. The final construct also contains the β-lactamase gene to confer ampicillin resistance for plasmid selection in *E. coli*. The sequence was validated by full length sequencing of each clone with greater than 1X coverage and a mutation rate of less than 0.2%.

Information related to the use of Gateway® Clones can be obtained from [Invitrogen™](#). A PCR product representing a functional hygromycin resistance cassette was assembled with chromosomal amplicons of approximately 600 base pairs of the regions flanking each gene targeted for replacement. The three fragments (left flank, hygromycin resistance gene, right flank) were amplified and cloned into pDONR™ entry vectors (Invitrogen™). Recombination was facilitated through an *attB* substrate (*attB*-PCR product or a linearized *attB* expression clone) with an *attP* substrate (pDONR™ vector) to create an *attL*-containing entry clone using the three-fragment [MultiSite Gateway® Pro](#) method. The hygromycin resistance cassette was sequence verified and experimentally verified through hygromycin resistance of DH10B-T1 *E. coli* cells. The final destination construct was confirmed by restriction digestion analysis. Please refer to the [Invitrogen™ Gateway® Technology Manual](#) for additional Gateway® product details.

Material Provided:

Every inoculated well of each 96-well plate contains approximately 60 µL of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) Broth containing 100 µg/mL ampicillin supplemented with 15% glycerol.

Note: Production in the 96-well format has increased risk of cross-contamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources cannot confirm or validate any clone not identified on the plate information tables found on the Product Information Sheets for the individual plates.

Packaging/Storage:

NR-19438 was packaged aseptically in a 96-well plate. The product is provided frozen and should be stored at -80°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:

LB Broth or Agar containing 100 µg/mL ampicillin.

Incubation:

Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 37°C.

Atmosphere: Aerobic

Propagation:

1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates at 37°C for 18 to 24 hours.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plates 1-8, NR-19438.”

Biosafety Level: 1

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.

Disclaimers:

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References:

1. Bardarov, S., et. al. "Specialized Transduction: an Efficient Method for Generating Marked and Unmarked Targeted Gene Disruptions in *Mycobacterium tuberculosis*, *M. bovis* BCG and *M. smegmatis*." Microbiology 148 (2002): 3007-3017. PubMed: 12368434.

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