

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

Catalog No. NR-19783

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Contributor:

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

Manufacturer:

BEI Resources

Product Description:

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 does not confirm or validate individual mutants provided by the contributor.

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%. Detailed information about each clone is shown in Table 1.

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:

Each inoculated well of the 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.

Packaging/Storage:

NR-19783 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*, Plate 1, NR-19783.”

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.

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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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Table 1: *Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clones, Plate 1 (KMTAA)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A01	MT0010	922447	NP_334418.1
A02	MT0022	922471	NP_334430.1
A03	MT0024	922477	NP_334432.1
A04	MT0030.1	922494	NP_334439.1
A05	MT0030	922490	NP_334438.1
A06	MT0032	922497	NP_334441.1
A07	MT0033	922499	NP_334442.1
A08	MT0036	922506	NP_334445.1
A09	MT0041	922598	NP_334450.1
A10	MT0044	922636	NP_334453.1
A11	MT0046	922638	NP_334454.1
A12	MT0049	922642	NP_334457.1
B01	MT0052	922655	NP_334460.1
B02	MT0053	922657	NP_334461.1
B03	MT0059	922720	NP_334467.1
B04	MT0060	922722	NP_334468.1
B05	MT0073	922801	NP_334483.1
B06	MT0083	922849	NP_334493.1
B07	MT0084	922855	NP_334494.1
B08	MT0087	922860	NP_334498.1
B09	MT0096	922898	NP_334505.1
B10	MT0098	922900	NP_334506.1
B11	MT0101	922913	NP_334510.1
B12	MT0102	922920	NP_334511.1
C01	MT0103	922921	NP_334512.1
C02	MT0104	922925	N/A
C03	MT0111	922960	NP_334519.1
C05	MT0114	922964	NP_334522.1
C06	MT0116.1	922967	NP_334525.1
C07	MT0125.1	922982	NP_334535.1
C08	MT0125	922981	NP_334534.1
C09	MT0127	922985	NP_334537.1
C10	MT0129	922989	NP_334539.1
C11	MT0130	922992	NP_334540.1
D01	MT0132	922994	NP_334542.1
D02	MT0133	922995	NP_334543.1

Well Position	Clone (MT Number)	Gene ID	Accession Number
D03	MT0138	923002	NP_334548.1
D04	MT0139	923004	NP_334549.1
D05	MT0140	923005	NP_334550.1
D06	MT0141	923006	NP_334551.1
D07	MT0142	923008	NP_334552.1
D08	MT0143	923009	NP_334553.1
D09	MT0144	923011	NP_334554.1
D11	MT0150	923021	NP_334560.1
D12	MT0158	923032	NP_334568.1
E01	MT0165	923039	NP_334574.1
E02	MT0170	923045	N/A
E03	MT0171	923046	NP_334578.1
E04	MT0174	923049	N/A
E05	MT0176	923051	NP_334582.1
E06	MT0192	923075	NP_334598.1
E07	MT0199	923084	NP_334605.1
E08	MT0205	923095	NP_334612.1
E09	MT0226	923122	NP_334633.1
E10	MT0234	923130	NP_334641.1
E11	MT0235	923131	NP_334642.1
E12	MT0238	923135	NP_334645.1
F01	MT0245	923143	NP_334651.1
F02	MT0250	923148	NP_334654.1
F03	MT0259	923165	NP_334663.1
F04	MT0265	923176	NP_334669.1
F05	MT0284	923213	NP_334689.1
F06	MT0285	923214	NP_334690.1
F08	MT0286	923215	NP_334691.1
F09	MT0291.4	923229	NP_334701.1
F10	MT0291	923224	NP_334697.1
F11	MT0294	923237	NP_334704.1
F12	MT0303	923252	NP_334713.1
G02	MT0311	923263	NP_334720.1
G03	MT0312	923264	NP_334721.1
G04	MT0319	923270	NP_334727.1
G05	MT0321	923282	NP_334729.1
G06	MT0327	923353	NP_334735.1
G07	MT0328.2	923391	NP_334738.1
G08	MT0329	923402	NP_334739.1
G09	MT0330	923405	NP_334740.1
G10	MT0333	923413	NP_334742.1
G11	MT0337	923434	NP_334746.1
H01	MT0353	923500	NP_334762.1
H02	MT0355	923502	NP_334763.1
H03	MT0359	923516	NP_334767.1
H04	MT0360	923518	NP_334768.1
H05	MT0365	923537	NP_334773.1
H06	MT0374	923559	NP_334781.1
H07	MT0379	923567	NP_334786.1
H08	MT0380	923570	NP_334787.1
H09	MT0391	923599	NP_334799.1
H10	MT0392	923601	NP_334800.1
H11	MT0393	923604	NP_334801.1
H12	MT0395	923607	NP_334803.1