Mycobacterium tuberculosis, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 5

Catalog No. NR-19787

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For research use only. Not for human use.

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

Manufacturer:
BEI Resources

Product Description:
Clone plates are replicated using a BioMek® FX robot. 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 only confirms the clone plate orientation and viability of randomly picked clones. BEI Resources does not confirm or validate individual clone identities 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 pYUB854[1] 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 *att*B substrate (*att*B-PCR product or a linearized *att*B expression clone) with an *att*P substrate (pDONR™ vector) to create an *att*L-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.

Plate orientation and viability were confirmed for NR-19787.

Material Provided:
Every 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-19787 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 containing 100 µg/mL ampicillin
LB agar containing 100 µg/mL ampicillin
Incubation:
Temperature: 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 5, NR-19787."

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/bmbl5/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.

Support Provided by NIAID

Table 1: *Mycobacterium tuberculosis*, Strain CDC1551, Knockout Gateway® Clones, Plate 5 (KMTAE)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A03	MT2180	924289	NP_336649.1
A05	MT2189	924263	NP_336659.1
A06	MT2195	924275	NP_336665.1
A07	MT2196	924268	NP_336666.1
A08	MT2197	924276	NP_336667.1
A09	MT2204	924258	NP_336674.1
A10	MT2220	924239	NP_336691.1
A11	MT2224	924235	NP_336695.1
A12	MT2225	924234	NP_336696.1
B01	MT2228	924231	NP_336699.1
B02	MT2233	924226	NP_336704.1
B03	MT2234	924225	NP_336705.1
B04	MT2237	924221	NP_336709.1
B05	MT2240	924193	NP_336712.1
B06	MT2241	924186	NP_336713.1
B07	MT2243	924183	NP_336715.1
B08	MT2248	924178	NP_336720.1
B09	MT2249	924177	NP_336721.1
B11	MT2259	924167	NP_336731.1
B12	MT2266	924159	NP_336738.1
C01	MT2267	924158	NP_336739.1
C02	MT2277	924149	NP_336748.1
C04	MT2284	924142	NP_336755.1
C05	MT2285	924141	NP_336756.1
C06	MT2287	924138	NP_336759.1
C07	MT2290	924136	NP_336760.1
C09	MT2296	924130	NP_336766.1
C10	MT2303	924123	NP_336773.1
C11	MT2308	924117	NP_336778.1
C12	MT2311	924114	NP_336781.1
D01	MT2312	924113	N/A
D02	MT2317	924109	NP_336785.1

Well Position	Clone (MT Number)	Gene ID	Accession Number
D03	MT2331	924093	NP_336800.1
D04	MT2332	924092	NP_336801.1
D05	MT2334.1	924089	NP_336804.1
D06	MT2339	924084	NP_336809.1
D07	MT2340	924083	NP_336810.1
D08	MT2344	924079	NP_336814.1
D09	MT2352	924069	NP_336823.1
D10	MT2356	924065	NP_336827.1
D11	MT2358	924063	NP_336829.1
D12	MT2362	924058	NP_336834.1
E01	MT2365.1	924053	NP_336839.1
E02	MT2365.2	924052	NP_336840.1
E03	MT2389	924023	NP_336867.1
E04	MT2390	924022	NP_336868.1
E05	MT2391	924021	NP_336869.1
E06	MT2393	924019	NP_336871.1
E07	MT2415	925938	NP_336894.1
E08	MT2417	924295	NP_336896.1
E09	MT2433	925918	NP_336913.1
E10	MT2454	925895	NP_336935.1
E11	MT2472	925864	NP_336954.1
E12	MT2474	925870	NP_336956.1
F01	MT2477	925866	NP_336959.1
F02	MT2490	925845	NP_336973.1
F03	MT2497	925838	NP_336980.1
F04	MT2511	925825	NP_336994.1
F05	MT2512	925824	NP_336995.1
F06	MT2513	925821	NP_336996.2
F08	MT2520.1	925812	NP_337005.1
F10	MT2526	925803	NP_337011.1
F11	MT2527	925804	NP_337012.1
F12	MT2533	925795	NP_337019.1
G01	MT2538	925777	NP_337024.1
G02	MT2547.1	925763	NP_337034.1
G03	MT2547.2	925785	NP_337035.1
G04	MT2552	925776	NP_337040.1
G05	MT2553	925775	NP_337041.1
G07	MT2581	925744	NP_337071.1
G08	MT2582	925746	NP_337072.1
G09	MT2584	925745	NP_337074.1
G10	MT2585	925736	NP_337075.1
G11	MT2586.1	925740	NP_337077.1
G12	MT2586	925738	NP_337076.1
H01	MT2587	925735	NP_337078.2
H02	MT2595	925717	NP_337088.1
H03	MT2597	925722	NP_337090.1
H05	MT2601	925715	NP_337095.1
H06	MT2603	925719	NP_337099.1
H07	MT2625	925693	NP_337122.1
H08	MT2626	925682	NP_337123.1
H11	MT2635	925680	NP_337133.1
H12	MT2655	925658	NP_337154.1

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