Mycobacterium tuberculosis, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 4

Catalog No. NR-19786

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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:

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 table.

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.

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-19786 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 4, NR-19786."

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.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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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 4 (KMTAD)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A01	MT1502	924466	NP_335952.1
A02	MT1506	924450	NP_335956.1
A03	MT1514.1	924451	NP_335965.1
A04	MT1523	924441	NP_335974.1
A05	MT1533	925512	NP_335984.1
A06	MT1536	924434	NP_335989.1
A07	MT1552	924419	N/A
A08	MT1555.1	924407	NP_336007.1
A09	MT1555	924406	NP_336006.1
A10	MT1557	924411	NP_336010.1
A11	MT1571	924385	NP_336024.1
A12	MT1578.1	924369	NP_336031.1
B01	MT1579	924370	NP_336032.1
B02	MT1583	924363	NP_336036.1
B03	MT1584	924364	N/A
B04	MT1585.1	924367	NP_336038.1
B05	MT1585	924366	NP_336037.1
B06	MT1586	924355	NP_336039.1
B07	MT1590	924360	NP_336042.1
B08	MT1601	924342	NP_336054.2
B09	MT1602	924343	NP_336055.1
B10	MT1607	924337	NP_336060.1
B12	MT1634	924296	NP_336088.1
C01	MT1642	924217	NP_336097.1
C02	MT1643	924218	NP_336098.1
C03	MT1662	924202	NP_336118.1
C04	MT1672	924196	NP_336128.1
C05	MT1679	923998	NP_336134.1
C07	MT1684	924339	NP_336139.1
C08	MT1697	924318	NP_336152.1
C09	MT1713	924412	NP_336167.1
C10	MT1714	924418	NP_336168.1
C11	MT1727	923982	NP_336181.1
C12	MT1746.1	923959	NP_336202.1

Well Position	Clone (MT Number)	Gene ID	Accession Number
D01	MT1760	923933	NP_336218.1
D02	MT1771	923936	NP_336229.1
D03	MT1772	923923	NP_336231.1
D04	MT1791	925926	NP_336250.1
D05	MT1794	923910	NP_336253.1
D06	MT1795	923909	NP_336254.1
D07	MT1796	923908	NP_336255.1
D08	MT1800	923901	NP_336259.1
D09	MT1801	923900	NP_336260.1
D10	MT1805	923895	NP_336264.1
D11	MT1812	923891	NP_336269.1
D12	MT1814	923887	NP_336271.1
E01	MT1817	923877	NP_336273.1
E02	MT1818	923875	N/A
E03	MT1823	923843	NP_336279.1
E04	MT1826	923825	NP_336282.1
E05	MT1828	923817	NP_336284.1
E06	MT1829	923815	NP_336285.1
E07	MT1830	923811	NP_336286.1
E08	MT1839.1	923240	NP_336297.1
E09	MT1843	923244	NP_336300.1
E11	MT1853	925137	NP_336310.1
E12	MT1854.1	925141	NP_336312.1
F01	MT1863	923769	NP_336322.1
F02	MT1877	923747	NP_336336.1
F03	MT1879	923746	NP_336337.1
F04	MT1880	923742	NP_336338.1
F05	MT1881	923741	NP_336339.1
F06	MT1885	923734	NP_336342.1
F07	MT1895	923717	NP_336352.1
F08	MT1902	923704	NP_336359.1
F09	MT1911	923687	NP_336368.1
F10	MT1916	923680	NP_336373.1
F11	MT1919	923674	NP_336376.1
F12	MT1929	923649	NP_336387.1
G01	MT1946	923619	NP_336403.1
G02	MT1957.1	923606	NP_336415.1
G03	MT1967	923063	NP_336425.1
G04	MT1971	923586	NP_336429.1
G05	MT1975	923574	NP_336433.1
G06	MT1976	923573	NP_336434.1
G07	MT1977	923572	NP_336435.1
G08	MT1995	923538	NP_336453.1
G09	MT1997	923536	NP_336454.1
G10	MT2015.1	923501	NP_336474.1
G11	MT2039	923399	NP_336500.1
G12	MT2041	923444	NP_336502.1
H01	MT2042	923442	NP_336503.1
H02	MT2054	923387	NP_336517.1
H03	MT2064	923372	NP_336527.1
H04	MT2066	923369	NP_336530.1
H06	MT2110	924635	NP_336575.1

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Well Position	Clone (MT Number)	Gene ID	Accession Number
H07	MT2113	924627	NP_336578.1
H08	MT2133	926388	NP_336599.1
H09	MT2134	924596	NP_336600.1
H11	MT2148	924582	N/A
H12	MT2151	924576	NP_336618.1

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