Product Information Sheet for NR-19785

Mycobacterium tuberculosis, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 3

Catalog No. NR-19785

This reagent is the tangible property of the U.S. Government.

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 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 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-19785 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 3, NR-19785."

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.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

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 3 (KMTAC)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A01	MT1024	925629	NP_335457.1
A02	MT1025.2	925174	NP_335460.1
A03	MT1031	925170	NP_335467.1
A06	MT1040.1	925160	NP_335477.1
A07	MT1040	925161	NP_335476.1
A08	MT1046	925154	NP_335483.1
A09	MT1055	925145	NP_335493.1
A10	MT1065	925101	NP_335502.1
A11	MT1068	925125	NP_335505.1
A12	MT1069	925124	NP_335506.1
B01	MT1072	925120	N/A
B02	MT1076	925115	NP_335514.2
B03	MT1079	925112	NP_335517.1
B04	MT1080	925111	NP_335518.1
B05	MT1093	925091	NP_335533.1
B07	MT1096.1	925089	NP_335537.1
B08	MT1111	925069	NP_335553.1
B09	MT1112	925070	NP_335554.1
B10	MT1118.1	925058	N/A
B11	MT1118	925064	NP_335560.1
B12	MT1123	924983	NP_335567.1
C01	MT1124	924980	NP_335568.1
C02	MT1125	924979	NP_335569.1
C03	MT1126	924977	NP_335570.1
C04	MT1132	924970	NP_335576.1
C05	MT1141	925400	NP_335584.1
C06	MT1147	924951	NP_335590.1
C07	MT1148	924950	NP_335591.1
C08	MT1156	924938	NP_335599.1
C09	MT1157.1	924935	NP_335600.1
C10	MT1157	924936	N/A
C11	MT1160	924932	NP_335603.1
C12	MT1165	924927	NP_335608.1
D01	MT1167	924924	NP_335610.1
D02	MT1169	924922	NP_335612.1

Well Position	Clone (MT Number)	Gene ID	Accession Number
D03	MT1174	924912	NP_335619.1
D04	MT1175	924911	NP_335620.1
D05	MT1180	924904	NP_335624.1
D07	MT1185	924896	NP_335628.1
D08	MT1195	924883	NP_335638.1
D10	MT1197	924880	NP_335640.1
D11	MT1203	924873	NP_335646.1
D12	MT1204	924872	NP_335647.1
E01	MT1207	924868	NP_335650.1
E02	MT1209	924865	NP_335652.1
E03	MT1210	924863	NP_335653.1
E04	MT1221	924851	NP_335664.1
E05	MT1231	924838	NP_335674.1
E07	MT1239	924828	NP_335682.1
E08	MT1240	924827	NP_335683.1
E09	MT1249	924821	NP_335692.1
E10	MT1252	924803	NP_335695.1
E12	MT1264.1	924801	NP_335708.1
F01	MT1280.1	923231	NP_335725.1
F02	MT1285	924786	NP_335729.1
F03	MT1303	924770	NP_335748.1
F04	MT1309	924761	NP_335755.1
F05	MT1313	924756	NP_335760.1
F06	MT1322	924749	NP_335769.1
F07	MT1329	924740	NP_335776.1
F08	MT1330.1	924741	NP_335778.1
F09	MT1335	924735	NP_335783.1
F10	MT1336	924733	NP_335784.1
F12	MT1353	923230	NP_335801.1
G01	MT1355	924705	NP_335803.1
G03	MT1366	924685	NP_335815.1
G04	MT1367	924716	NP_335816.1
G05	MT1389	924640	NP_335839.1
G06	MT1395	924621	NP_335844.1
G07	MT1404	924595	NP_335853.1
G08	MT1418.1	924564	NP_335869.1
G09	MT1437	924711	NP_335888.1
G10	MT1440.1	924532	N/A
G11	MT1445	924527	NP_335896.1
G12	MT1446	924526	NP_335897.1
H01	MT1447	924525	NP_335898.1
H02	MT1456	924516	NP_335907.1
H03	MT1457	924515	NP_335908.1
H04	MT1462	924509	NP_335913.1
H06	MT1479.1	924492	NP_335930.1
H07	MT1483	924488	N/A
H08	MT1485	924486	NP_335935.1
H09	MT1486	924485	N/A
H10	MT1497.2	924468	NP_335947.1
H11	MT1498	924471	NP_335948.1
H12	MT1499	924473	NP_335949.1

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898