Product Information Sheet for NR-19789

Mycobacterium tuberculosis, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 7

Catalog No. NR-19789

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-19789 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 7, NR-19789."

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 7 (KMTAG)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A01	MT3256	923336	NP_337781.1
A03	MT3260	923332	NP_337785.1
A04	MT3261	923331	NP_337786.1
A05	MT3262	923330	NP_337787.1
A06	MT3265	923327	NP_337790.1
A07	MT3286	923298	NP_337813.1
A08	MT3303	923273	NP_337831.1
A09	MT3304	923272	NP_337832.1
A10	MT3305	923853	NP_337833.1
A11	MT3309	922979	NP_337837.1
A12	MT3323.1	924682	NP_337852.1
B01	MT3327	922787	NP_337856.1
B02	MT3332	922646	NP_337861.1
B03	MT3339	922876	NP_337867.1
B04	MT3344	923067	NP_337872.1
B05	MT3345	923013	NP_337873.1
B06	MT3346	922442	NP_337874.1
B07	MT3356	923978	NP_337884.1
B08	MT3357	923180	NP_337885.1
B09	MT3358	923243	NP_337886.1
B10	MT3371	922436	NP_337898.1
B11	MT3377.1	922459	NP_337905.1
B12	MT3377	922472	NP_337904.1
C01	MT3379	922463	NP_337907.1
C02	MT3384	922487	NP_337913.1
C03	MT3393	922502	NP_337922.1
C04	MT3401	926639	NP_337930.1
C05	MT3402	926621	NP_337931.1
C06	MT3403	926277	NP_337932.1
C07	MT3408	926292	NP_337937.1
C08	MT3413	926307	NP_337942.1
C09	MT3414	922565	N/A
C10	MT3432	926352	NP_337961.1
C11	MT3435	926401	NP_337964.1

Well Position	Clone (MT Number)	Gene ID	Accession Number
C12	MT3437	926403	NP_337966.1
D01	MT3441	926415	NP_337971.1
D04	MT3459	926464	NP_337984.1
D05	MT3475	926503	NP_337999.1
D06	MT3476	926500	NP_338000.1
D07	MT3477	926518	NP_338001.1
D08	MT3484	926536	N/A
D09	MT3486	926531	NP_338008.1
D10	MT3496	926565	NP_338019.1
D11	MT3499	926573	NP_338022.1
D12	MT3501	926571	NP_338024.1
E01	MT3502	926574	NP_338025.1
E02	MT3507	926591	NP_338030.1
E03	MT3513	922924	NP_338037.1
E04	MT3521	922958	NP_338045.1
E05	MT3542	923526	NP_338068.1
E06	MT3546	922957	NP_338073.1
E07	MT3578	922928	NP_338121.1
E08	MT3579	922915	NP_338122.1
E09	MT3612.1	922888	NP_338157.1
E10	MT3615.3	922877	NP_338160.1
E11	MT3617	926581	NP_338163.1
E12	MT3618	922880	NP_338164.1
F01	MT3619	926584	NP_338165.1
F02	MT3624	922870	NP_338171.1
F03	MT3627	922872	NP_338174.1
F04	MT3629	922862	NP_338176.1
F05	MT3643	922858	NP_338188.1
F06	MT3650	922843	NP_338195.1
F07	MT3659	922844	N/A
F08	MT3678	926537	NP_338223.1
F09	MT3685	926535	NP_338229.1
F10	MT3690	926540	NP_338234.1
F11	MT3693	926529	NP_338237.1
F12	MT3696	922794	NP_338240.1
G01	MT3697	922807	NP_338241.1
G02	MT3700	922791	NP_338244.1
G03	MT3701	926532	NP_338245.1
G04	MT3703	922793	NP_338246.1
G05	MT3704	926522	NP_338247.1
G06	MT3716	922780	NP_338261.1
G07	MT3717	926517	NP_338262.1
G08	MT3725	922766	NP_338272.1
G09	MT3730	922764	NP_338277.1
G10	MT3736	926502	NP_338283.1
G11	MT3737	922757	NP_338284.1
G12	MT3740	922752	NP_338285.1
H01	MT3746	922751	NP_338292.1
H02	MT3747	922753	NP_338293.1
H03	MT3748	926496	NP_338294.1
H04	MT3749	922741	NP_338295.1
H05	MT3750	926495	NP_338296.1
H06	MT3756	926481	NP_338303.1
H07	MT3757	926488	NP_338307.1

Well Position	Clone (MT Number)	Gene ID	Accession Number
H08	MT3760	926478	NP_338310.1
H09	MT3770	922716	NP_338323.1
H10	MT3771	922727	NP_338324.1
H12	MT3789	922695	NP_338342.1

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