Product Information Sheet for NR-19788

Mycobacterium tuberculosis, Strain CDC1551, Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, Plate 6

Catalog No. NR-19788

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-19788 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 6, NR-19788."

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 6 (KMTAF)

Well Position	Clone (MT Number)	Gene ID	Accession Number
A01	MT2659	925654	NP_337158.1
A02	MT2678	925631	NP_337180.1
A03	MT2682	925628	NP_337184.1
A04	MT2689.1	925613	N/A
A05	MT2690	925614	NP_337192.1
A06	MT2691.1	925616	NP_337193.1
A07	MT2697	925607	NP_337199.1
A08	MT2703	925594	NP_337205.1
A09	MT2715	925595	NP_337215.1
A10	MT2716	925491	NP_337216.1
A11	MT2725	925573	NP_337225.1
A12	MT2729	925572	NP_337229.1
B01	MT2756	925530	NP_337257.1
B02	MT2759	925539	NP_337260.1
B03	MT2763	925533	NP_337264.1
B04	MT2769	925514	NP_337270.1
B05	MT2770	925534	NP_337271.1
B06	MT2771	925523	NP_337272.1
B07	MT2772	925525	NP_337273.1
B08	MT2774	925527	NP_337275.1
B09	MT2775	925526	NP_337276.1
B10	MT2783	925516	NP_337285.1
B11	MT2784	925513	NP_337286.1
B12	MT2792	925504	NP_337294.1
C01	MT2793	925502	NP_337295.1
C02	MT2802.1	925487	NP_337307.1
C03	MT2803	925473	NP_337308.1
C04	MT2813	925472	NP_337319.1
C05	MT2817	925460	NP_337323.1
C07	MT2824	925465	NP_337329.1
C08	MT2833	925455	NP_337338.1
C09	MT2834	925453	NP_337339.1
C10	MT2836	925450	NP_337341.1
C11	MT2851	925434	NP_337356.1
C12	MT2856	925426	NP_337361.1
D01	MT2869	925411	NP_337376.1

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Well Position	Clone	Gene ID	Accession Number
D03	MT2873	925407	NP_337380.1
D04	MT2874	925406	NP_337381.1
D05	MT2875	925405	NP_337382.1
D07	MT2880.3	924901	NP_337389.1
D08	MT2897	925377	NP_337409.1
D09	MT2906	925369	NP_337418.1
D10	MT2911	925364	NP_337423.2
D11	MT2923	925356	NP_337434.1
D12	MT2924	925353	NP_337435.1
E01	MT2929	925347	NP_337440.1
E02	MT2939	925337	NP_337450.1
E03	MT2951	925329	NP_337462.2
E04	MT2955	925324	NP_337466.1
E05	MT2961	925318	NP_337473.1
E06	MT2983	925292	NP_337495.1
E07	MT2984	925291	NP_337496.1
E08	MT2989	925285	NP_337502.1
E09	MT2997	925279	NP_337510.1
E10	MT3016	925261	NP_337529.1
E11	MT3038	925238	NP_337550.1
E12	MT3044	925232	NP_337556.1
F02	MT3049	925221	NP_337561.1
F03	MT3059	925214	NP_337574.1
F04	MT3061	925211	NP_337576.1
F05	MT3064	925208	NP_337579.1
F06	MT3067	925205	NP_337582.1
F07	MT3069	925191	NP_337584.1
F08	MT3072	925201	NP_337587.1
F09	MT3075	925198	NP_337590.1
F10	MT3080	926682	NP_337594.1
F11	MT3086	923608	NP_337601.1
F12	MT3094	923055	NP_337609.2
G02	MT3096	923220	NP_337611.1
G03	MT3107	926358	NP_337623.1
G04	MT3119	923211	NP_337635.1
G05	MT3120	923087	NP_337636.1
G06	MT3129	922594	NP_337645.1
G07	MT3130	923169	NP_337646.1
G08	MT3132	922595	NP_337649.1
G09	MT3133	922702	NP_337650.1
G10	MT3140	923094	NP_337658.1
G11	MT3144	923254	NP_337662.1
G12	MT3152	926720	NP_337673.1
H02	MT3180	926705	NP_337704.1
H03	MT3191	926668	NP_337716.1
H04	MT3197	926687	NP_337723.2
H05	MT3212	923449	NP_337738.1
H06	MT3214	923446	
H07	MT3217	923427	NP_337741.1
H08	MT3221	923408	NP_337747.1
H09	MT3222	923407	NP_337748.1
H10	MT3228	923397	NP_337754.1
H11	MT3235	923382	NP_337760.1
H12	MT3239	923368	NP_337764.1