H1N1pdm09 Gateway® Clone Set, Recombinant in *Escherichia coli*

Catalog No. NR-19271

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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 H1N1pdm09 Gateway® Clone Set (also 2009 H1N1 Gateway® Clone Set) contains Influenza A (H1N1)pdm09 open reading frames from two clinical isolates A/New York/1682/2009 (H1N1)pdm09 (CY039901 to CY039908) and A/New York/1669/2009 (H1N1)pdm09 (CY039893 to CY039900) cloned in *Escherichia coli* (*E. coli*) DH10B or Stbl4 cells. The clone set consists of twenty-seven clones that were constructed in vector pDONR™221. The full annotated coding sequence (CDS) for each genomic segment has been cloned (stop codons removed) and the sequence verified. The transmembrane regions of the hemagglutinin and neuraminidase coding sequences have been truncated. Detailed information about each clone is shown in Table 1.

Information related to the use of Gateway® Clones can be obtained from 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™221) to create an *att*L-containing entry clone. The entry clone contains recombinational cloning sites, *att*L1 and *att*L2 to facilitate gene transfer into a destination vector, M13 forward and reverse priming sites for sequencing and a kanamycin resistance gene for selection. Please refer to the Invitrogen™ Gateway® Technology Manual for additional details.

Plate orientation and viability were confirmed for NR-19271.

Material Provided:

Each well of the 96-well plate contains approximately 50 µL of *E. coli* culture (strain DH10B-T1 or strain Stbl4, see Table 1 for culture details) in Luria Bertani (LB) broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

Packaging/Storage:

NR-19271 was packaged aseptically in 96-well plates. 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 50 µg/mL kanamycin
LB agar containing 50 µg/mL kanamycin
Incubation:
Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 37°C and *E. coli*, strain Stbl4 clones which should be grown at 30°C.
Atmosphere: Aerobic
Propagation:

1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates at the temperatures indicated above. All clones should be grown for 18 to 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: H1N1pdm09 Gateway® Clone Set, Recombinant in *Escherichia coli*, NR-19271."

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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Support Provided by NIAID

Table 1: H1N1pdm09 Gateway® Clone Set, Recombinant in *Escherichia coli*

Clone	Well Position	ORF Lengths	Description	Average Depth of Coverage	Class[1]	Cell Type	Growth Temperature
79143	A01	331	CDS(M2)_MP-NY1682	5.5	A	DH10B	37°C
79114	A02	733	CDS(NS1)_NS-NY1682	7.0	A	DH10B	37°C
79154	A03	933	NS-seg8-NY1682	8.1	CSPT	DH10B	37°C
79094	A04	1070	MP-seg7-NY1682	8.3	CSPT	DH10B	37°C
79177	A05	1366	CDS(-TM)_NA-NY1682	8.6	BSC	DH10B	37°C
79147	A06	1450	wholeCDS_NA-NY1682	8.2	BSC	DH10B	37°C
79186	A07	1501	NA-seg6-NY1682	8.1	CSPT	DH10B	37°C
79140	A08	2191	CDS_PA-NY1682	5.5	A	DH10B	37°C
79179	A09	2320	CDS_PB2-NY1682	8.4	BLM	DH10B	37°C
79067	A10	331	CDS(M2)_MP-NY1669	5.7	A	DH10B	37°C
79032	A11	406	CDS(NS2)_NS-NY1669	5.3	A	DH10B	37°C
79035	A12	1070	MP-seg7-NY1669	8.3	CSPT	DH10B	37°C
79039	B01	1608	NP-seg5-NY1669	8.6	CSPT	DH10B	37°C
79263	B02	406	CDS(NS2)_NS-NY1682	5.0	A	DH10B	37°C
79294	B03	799	CDS(M1)_MP-NY1682	6.9	A	DH10B	37°C
79302	B04	1821	HA-seg4-NY1682	8.5	CFC	DH10B	37°C
79287	B05	799	CDS(M1)_MP-NY1669	6.9	A	DH10B	37°C
79215	B06	933	NS-seg8-NY1669	8.0	CSPT	DH10B	37°C
81821	B07	733	CDS(NS1)_NS-NY1682	2.0	A	DH10B	37°C
82466	B08	1608	NP-seg5-NY1682	7.4	CSPT	Stbl4	30°C
82469	B09	1633	CDS(-TM)_HA-NY1682	7.7	CFM	Stbl4	30°C
82475	B10	1741	wholeCDS_HA-NY1682	6.0	BLM	Stbl4	30°C
82481	B11	2314	CDS_PB1-NY1682	5.5	BLM	Stbl4	30°C
82485	B12	733	CDS(NS1)_NS-NY1669	5.1	A	Stbl4	30°C
82489	C01	2384	PB2-seg1-NY1682	5.4	CSPT	Stbl4	30°C
82494	C02	1450	wholeCDS_NA-NY1669	5.2	A	Stbl4	30°C
82500	C03	1501	NA-seg6-NY1669	6.7	CSPT	Stbl4	30°C

[1] **A:** Full-length sequence validation, 2X or greater coverage, 100% sequence identity with the reference ORF.
B: Full-length sequence validation, sequence variation (less than 100% sequence identity with the reference ORF); remains valid.
BLM: B class clone with substitutions in CDS only at ≤ 0.2% mutation rate.
BSC: B class clone with substitutions in CDS only leading to silent mutations.
C: Full-length sequence validation, sequence variation (less than 100% sequence identity with the reference ORF); becomes invalid.
CFC: C class clone with frameshift mutations in CDS only.
CFM: C class clone with frameshift mutations in two or more regions (ATT, CDS, and stop codon) of the validated sequence.
CSPT: C class clone with substitution resulting in truncated protein (nonsense mutation).

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