

SUPPORTING INFECTIOUS DISEASE RESEARCH

Product Information Sheet for NR-19435

H1N1pdm09 Expression Clone Set, Recombinant in Escherichia coli

Catalog No. NR-19435

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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 crosscontamination 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 H1N1pdm09 Expression Clone Set (also 2009 H1N1 Expression 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 cells. The clone set consists of thirty clones that were constructed in either pIVE-LIC-His cHalo, or the Promega vectors pFC20A or pFC14A. The full annotated coding sequence (CDS) for each genomic segment has been cloned (stop codons removed) and the sequence verified. The hemagglutinin CDS was truncated to remove the 3' transmembrane domain and the tailing sequence and the neuraminidase CDS was spliced to remove the 5' transmembrane domain but retain the 5' eighteen base pair leader sequence.

Detailed information about each clone is shown in Table 1. Information related to the use of the expression vector can be obtained from Promega.

Material Provided:

Each well of the 96-well plate contains approximately 60 μ L of *E. coli* culture (strain DH10B) in Luria Bertani (LB) broth containing 100 μ g/mL ampicillin supplemented with 15% alycerol.

Packaging/Storage:

NR-19435 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 agar containing 100 µg/mL ampicillin

Incubation:

Temperature: E. coli, strain DH10B clones should be grown

at 37°C in a shaking incubator at 225 rpm

Atmosphere: Aerobic

Propagation:

- 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: H1N1pdm09 Expression Clone Set, Recombinant in Escherichia coli, NR-19435."

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. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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Table 1: H1N1pdm09 Expression Clones

Clone	Well Position	ORF Length	Description	Average Depth of Coverage	Class ¹	Cell Type	Vector Type
	A01	792		9.2	А	DH10B	pIVE-LIC-
10043			CDS(M1)_MP-NY1682				cHalo
	A02	324	, ,=	7.7	Α	DH10B	pIVE-LIC-
10020			CDS(M2)_MP-NY1682				cHalo
	A03	399		7.3	BLM	DH10B	pIVE-LIC-
10030			CDS(NS2)_NS-NY1669				cHalo
	A04	399		7.3	Α	DH10B	pIVE-LIC-
10041			CDS(NS2)_NS-NY1682				cHalo
	A05	2307		7.5	BLM	DH10B	pIVE-LIC-
10609			CDS_PB1-NY1682				cHalo
10562	A06	1623		5.6	BLM	DH10B	pIVE-LIC-
			CDS(-TM)_HA-NY1682				cHalo
10579	A07	1374		6.6	BSC	DH10B	pIVE-LIC-
			CDS_NA_splice-NY1682				cHalo
	A08	726		4.1	Α	DH10B	pIVE-LIC-
10565			CDS(NS1)_NS-NY1669				cHalo
10646	A09	2184		9.6	Α	DH10B	pIVE-LIC-
	7.00	2.0.	CDS_PA-NY1682				cHalo
	A10	726		-	V	DH10B	pIVE-LIC-
10564			CDS(NS1)_NS-NY1669				cHalo
10695	A11	2327	CDS_PB2-NY1682	7.9	BLM	DH10B	pFC20A
10756	A12	2321	CDS_PB1-NY1682	7.5	BLM	DH10B	pFC20A
10697	B01	1637	CDS(-TM)_HA-NY1682	8.0	CFC	DH10B	pFC20A
10194	B02	1388	CDS_NA_splice-NY1669	8.1	BLM	DH10B	pFC20A
10728	B03	1388	CDS_NA_splice-NY1682	7.0	BSC	DH10B	pFC20A
10193	B04	806	CDS(M1)_MP-NY1669	7.0	Α	DH10B	pFC20A
10310	B05	338	CDS(M2)_MP-NY1669	3.7	Α	DH10B	pFC20A
10272	B06	707	CDS(NS1)_NS-NY1669	5.9	Α	DH10B	pFC20A
10700	B07	413	CDS(NS2)_NS-NY1682	5.3	A	DH10B	pFC20A
10394	B08	1388	CDS_NA_splice-NY1669	7.9	Α	DH10B	pFC14A
10373	B09	806	CDS(M1)_MP-NY1669	5.8	Α	DH10B	pFC14A
10831	B10	2321	CDS_PB1-NY1682	6.9	BLM	DH10B	pFC14A
10920	B11	2327	CDS_PB2-NY1682	8.0	CSPT	DH10B	pFC14A
10820	B12	413	CDS(NS2)_NS-NY1682	5.3	Α	DH10B	pFC14A
10818	C01	1388	CDS_NA_splice-NY1682	6.9	BSC	DH10B	pFC14A
10092	C02	740	CDS(NS1)_NS-NY1669	5.1	D	DH10B	pFC14A
10090	C03	338	CDS(M2)_MP-NY1669	3.5	Α	DH10B	pFC14A
12035	C04	1544	CDS_NP-NY1669	3.6	BLM	DH10B	pFC14A
12043	C05	1637	CDS(-TM)_HA-NY1669	4.9	CFC	DH10B	pFC14A
12044	C06	1637	CDS(-TM)_HA-NY1669	3.7	CFC	DH10B	pFC14A

¹A: Full-length sequence validation, 2X or greater coverage, 100% sequence identity with the reference ORF.

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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 $\leq 0.2\%$ 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.

CSPT: C class clone with substitution resulting in truncated protein (nonsense mutation).

D: Partial sequence validation, single contig with missing end-sequence (less than 90% sequence identity with the reference ORF).