SARS Coronavirus, Tor2, Complete Gateway® Clone Set, Recombinant in *Escherichia coli*

Catalog No. NR-19270

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Contributor:

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

Product Description:

SARS Coronavirus, Tor2, Gateway® clones were designed for features based on the annotation from the GenBank entry AY274119 and the corresponding RefSeq entry for NC_004718. The sequences from both entries are identical but there are annotation differences. The clones were designed from the annotated ORFs from AY274119 and from the annotated protein coding regions of NC_004718. The clone set consists of twenty-seven clones that were constructed in vector pDONR 221 (Invitrogen). ATG start codons were added to the forward primer sequences when required and stop codon sequence was trimmed from the reverse primer sequences. Each clone has been sequenced using a combination of end sequencing and primer walking to determine each base at an average of 2-fold coverage.

Detailed information about each clone is shown in Table 1. Information related to the use of Gateway® Clones can be obtained from Invitrogen.

Material Provided:

Each well of the 96-well plate contains approximately 40 µL of *Escherichia coli* culture [strain DH10B-T1, or strain Stbl for clones NC_828851, NC_828869(a) and NC_828869(b)] in Luria Bertani (LB) Broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

Note: 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 cannot confirm or validate any clone not identified on the plate information table.

Packaging/Storage:

NR-19270 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 or Agar containing 25 µg/mL kanamycin

Incubation:
Temperature: Clones should be grown at 37°C except for clones NC_828851, NC_828869(a) and NC_828869(b), 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, except for clones NC_828851, NC_828869 and NC_828869b, which should be incubated for 16 to 18 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: SARS Coronavirus, Tor2, Complete Gateway® Clone Set, Recombinant in *Escherichia coli*, NR-19270."

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:

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Table 1: SARS Coronavirus, Tor2, Gateway® Clones

Clone (Acession Number)	Well Position	Coordinates	Description	Class[1]
NP_828860	A01	265…801	Leader protein nsp1-pp1/pp1ab	A
NP_828861	A02	802…2718	Counterpart of MHV p65 protein nsp2-pp1a/pp1ab	C
NP_828863	A03	9985…10902	3C-like proteinase nsp5-pp1a/pp1ab (3CL-PRO)	B
NP_828864	A04	10903…11772	Transmembrane protein nsp6-pp1a/pp1ab	B
NP_828865	A05	11773…12021	Protein nsp7-pp1a/pp1ab	B
NP_828866	A06	12022…12615	Protein nsp8-pp1a/pp1ab	A
NP_828867	A07	12616…12954	RNA-binding protein nsp9-pp1a/pp1ab	A
NP_828868	A08	12955…13371	Protein nsp10-pp1a/pp1ab	A
NP_828869(a)	A09	13372…16166	RNA-dependent RNA polymerase nsp12-pp1ab (RdRp) (with leader)	C
NP_828869(b)	A10	13398…16166	RNA-dependent RNA polymerase nsp12-pp1ab (RdRp) (without leader)	C
NP_828870	A11	16167…17969	Zinc-binding NTPase/helicase nsp13-pp1ab (ZD NTPase/HEL)	A
NP_828871	A12	17970…19550	nsp14-pp1ab (nuclease ExoN homolog)	A
NP_828872	B01	19551…20588	Replicative endoribonuclease NendoU nsp15-pp1ab	A
NP_828873	B02	20589…21482	Ribose 2'-O-methyltransferase nsp16-pp1ab	B
NP_828851	B03	21492…25259	E2 glycoprotein precursor; putative spike glycoprotein	C
NP_828852	B04	25268…26092	Protein 3a (sars3a)	B
NP_828853	B05	25689…26153	Putative protein 3a (sars3b)	B
NP_828854	B06	26117…26347	Small envelope protein E	B
NP_828855	B07	26398…27063	Matrix protein M	B
NP_828856	B08	27074…27265	Putative protein (sars6)	B
NP_828857	B09	27273…27641	Putative protein (sars7a)	A
NP_849175	B10	27638…27772	Putative protein (sars7b)	C
NP_849176	B11	27779…27898	Putative protein (sars8a)	B
NP_849177	B12	27864…28118	Putative protein (sars8b)	B
NP_828858	C01	28120…29388	Nucleocapsid protein N	A
NP_828859	C02	28130…28426	Putative protein (sars9b)	B
AAP41049	C03	28583…28795	Orf14 (SARS coronavirus, Tor2)	B

[1]**Class A:** 100% identity to target with >= 2X coverage over the full length of the insert. These clones should be considered completely error free.
Class B: 100% identity to target with < 2X coverage over the full length of the insert. These clones have no mismatches relative to the intended insert sequence, however there are regions where we have only been able to verify the sequence using one read.
Class C: Less than 100% identity to intended target sequence. These clones either have not been completely verified, or have sequence that differs from the Genbank Accession.
Class D: These clones have not yet been completely verified, due to sequencing gaps.