Certificate of Analysis for NR-53260

Plasmid Set for Anti-SARS Coronavirus Human Monoclonal Antibody CR3022

Catalog No. NR-53260

This reagent is the tangible property of the U.S. Government.

Product Description:

The vectors for human anti-severe acute respiratory syndrome coronavirus (SARS-CoV) immunoglobulin heavy and light chain variable regions (CR3022 vH and vL, GenBank: DQ168569 and DQ168570, respectively) were designed for transient expression of recombinant SARS-CoV neutralizing antibody CR3022 when used in combination. The CR3022 vH and vL sequences were subcloned into mammalian expression vectors (pFUSEss-CHIg-hG1 and pFUSEss-CLIg-hk, respectively) fused to the N-terminal interleukin 2 (IL2) signal sequence and the C-terminal constant regions of human IgG1 (hIgG1) heavy or human Ig kappa (hIgκ) light chain. NR-52399 contains the *Streptoalloteichus hindustanus* bleomycin *(Sh ble)* gene to provide transformant selection through Zeocin™ resistance in *Escherichia coli (E. coli)* and mammalian cells. NR-52400 contains the *Bacillus cereus* blasticidin-S deaminase *(bsr)* gene to provide transformant selection through blasticidin resistance in *E. coli* and mammalian cells. Each deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with the appropriate antibiotic (NR-52399: 50 µg per mL Zeocin™; NR-52400: 100 µg per mL blasticidin) for 1 day at 37°C in an aerobic atmosphere, extracted using a QIAGEN® Plasmid *Plus* Maxi Kit and vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Vector pFUSEss-CHIg-hG1 Containing CR3022 (Heavy Chain), Human Monoclonal Anti-SARS-CoV
NR-52399 lot 70034830 (Mfg. Date: 20APR2020)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	~ 4830 base pairs	4833 base pairs[1]
Genotypic Analysis Sequencing of CR3022 vH insert (~ 350 base pairs) Sequencing of pFUSEss-CHIg-HG1 vector	≥ 99% sequence identity to CR3022 (GenBank: DQ168569.1) N-terminal IL2 signal sequence C-terminal hIgG1 heavy constant region	100% sequence identity to CR3022 (GenBank: DQ168569.1) N-terminal IL2 signal sequence C-terminal hIgG1 heavy constant region
Antibiotic Resistance Zeocin™ (encoded by *Sh ble* gene)[2]	*Sh ble* sequence present	*Sh ble* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg per mL	0.3 µg in 20 µL per vial (13 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	89 colonies per ng

[1]The sequence was assembled using the depositor's predicted sequence as the reference sequence.
[2]The antibiotic Zeocin™ is light sensitive, and high ionic strength or extreme pH inhibits its activity. Reducing the salt in the bacterial medium and adjusting the pH to 7.5 is recommended to keep the drug active.

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Vector pFUSEss-CLIg-hk Containing CR3022 (Light Chain), Human Monoclonal Anti-SARS-CoV
NR-52400 lot 70035737 (Mfg. Date: 07MAY2020)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	~ 4190 base pairs	4193 base pairs[3]
Genotypic Analysis Sequencing of CR3022 vL insert (~ 340 base pairs) Sequencing of pFUSEss-CLIg-hk vector	≥ 99% sequence identity to CR3022 (GenBank: DQ168570.1) N-terminal IL2 signal sequence C-terminal hIgκ light constant domain	100% sequence identity to CR3022 (GenBank: DQ168570.1) N-terminal IL2 signal sequence C-terminal hIgκ light constant domain
Antibiotic Resistance Blasticidin (encoded by *bsr* gene)	*bsr* sequence present	*bsr* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg per mL	0.5 µg in 20 µL per vial (23 µg/mL)
Amount per Vial	Report results	0.5 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	59 colonies per ng

[3]The sequence was assembled using the depositor's predicted sequence as the reference sequence.

/Heather Couch/
Heather Couch 28 MAY 2020
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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