

# **Certificate of Analysis for NR-52725**

### **Human Coronavirus, OC43**

### Catalog No. NR-52725

### **Product Description:**

Human coronavirus (HCoV), OC43 was isolated in 1967 from a respiratory sample from a human adult with a cold-like illness in the Common Cold Unit, Salisbury, England, United Kingdom and deposited with ATCC® as VR-759™, which was cleaned of mycoplasma contamination and adapted to cell culture and became VR-1558™. VR-1558 was used to produce BEI Resources NR-52725. NR-52725 lot 70035625 was produced by infecting human ileocecal colorectal adenocarcinoma cells (HCT-8 [HRT-18]; ATCC CCL-244™) and incubating in RPMI-1640 medium (ATCC 30-2001™) supplemented with 2% heat-inactivated horse serum (Gibco® 26050-088) for 6 days rocking at 33°C with 5% CO₂.

#### Passage History:

X(?)HRT-18(7)/HCT-8(6)/HCT-8(1) (Prior to deposit at ATCC/ATCC/BEI Resources); X = Unknown; HRT-18 = Human ileocecal colorectal adenocarcinoma cells; HCT-8 = Human ileocecal colorectal adenocarcinoma cells (ATCC CCL-244)

Lot: 70035625 Manufacturing Date: 13MAY2020

| TEST  | SPECIFICATIONS   | RESULTS  |
|---|--|--|
| Identification by Infectivity in HCT-8 Cells  | Cell vacuolization and sloughing                           | Cell vacuolization and sloughing                           |
| Whole Genome Sequencing (~ 307200 nucleotides)  | ≥ 98% identity with HCoV,<br>OC43 (GenBank:<br>AY585228.1) | 99.7% identity with HCoV,<br>OC43 (GenBank:<br>AY585228.1) |
| Titer by TCID <sub>50</sub> Assay in HCT-8 Cells by Cytopathic Effect <sup>1</sup> (11 days at 33°C with 5% CO <sub>2</sub> ) | Report results   | 8.9 × 10 <sup>4</sup> TCID <sub>50</sub> per mL            |
| Amplification of HCoV Sequence by RT-PCR  | ~ 430 base pair amplicon                                   | ~ 430 base pair amplicon                                   |
| Sequencing of Species-Specific Region<br>(~ 410 nucleotides)  | ≥ 98% identity with HCoV,<br>OC43 (GenBank:<br>AY585228.1) | 99.8% identity with HCoV,<br>OC43 (GenBank:<br>AY585228.1) |
| Sterility (22-day incubation)   |  |  |
| Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup>   | No growth  | No growth  |
| Trypticase Soy broth, 37°C and 26°C, aerobic  | No growth  | No growth  |
| Sabouraud broth, 37°C and 26°C, aerobic   | No growth  | No growth  |
| Sheep blood agar, 37°C, aerobic   | No growth  | No growth  |
| Sheep blood agar, 37°C, anaerobic   | No growth  | No growth  |
| Thioglycollate broth, 37°C, anaerobic   | No growth  | No growth  |
| DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>  | No growth  | No growth  |
| Mycoplasma Contamination  |  |  |
| Agar and broth culture (14-day incubation at 37°C)  | None detected  | None detected  |
| DNA detection by PCR of extracted Test Article nucleic acid   | None detected  | None detected  |

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.
²Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

## /Heather Couch/

Heather Couch 06 JUL 2020

Program Manager or designee, ATCC Federal Solutions

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898