

Certificate of Analysis for NR-51391

Human Astrovirus Type 4, Oxford

Catalog No. NR-51391

Product Description:

Human astrovirus type 4 (HAstV4), Oxford was isolated from the stool of a human with acute gastroenteritis in Oxford, United Kingdom.

Passage History:

X/C3 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; C = CaCO-2 cells¹

Lot: 70025946² Manufacturing Date: 30JUL2019

| TEST | SPECIFICATIONS | RESULTS |
|--|---|---|
| Identification by Infectivity in CaCO-2 Cells ¹ | Report results | Cell rounding and detachment |
| Identification by Indirect Fluorescent Antibody (IFA) Assay³ | Fluorescence observed | Fluorescence observed |
| Sequencing of Species-Specific Region (~ 360 nucleotides) | ≥ 98% identity with HAstV4, Oxford (GenBank: MK059952.1) | 100% identity with HAstV4, Oxford (GenBank: MK059952.1) |
| Titer by TCID₅₀ Assay in CaCO-2 cells with IFA Readout¹,₃,₄,₅ | Report results | 1.6 × 10 ⁷ TCID ₅₀ per mL |
| Amplification of HAstV4 Capsid Sequence by RT-PCR | ~ 450 base pair amplicon | ~ 450 base pair amplicon |
| Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂ | No growth | No growth |
| Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid | None detected None detected | None detected None detected |

¹Human colon adenocarcinoma cells (CaCO-2; ATCC® HTB-37™)

/Heather Couch/

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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.

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²Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (EMEM; ATCC[®] 30-2003) supplemented with 1 μg/mL trypsin type IX-S for 2 days at 37°C with 5% CO₂. Virus was activated by incubating with 5 μg/mL trypsin type IX-S in EMEM for 30 minutes at 37°C before infecting the cells.

³Using monoclonal Astrovirus Type 1-5, Člone J12H (ThermoFisher Scientific™ MA5-18174)

⁴The 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.

⁵Assay plates were incubated 5 days at 37°C and 5% CO₂.

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.