

Certificate of Analysis for NR-51390

Human Astrovirus Type 3, Oxford

Catalog No. NR-51390

Product Description:

Human astrovirus type 3 (HAstV3), Oxford was isolated from the stool of a human with acute gastroenteritis in Oxford, United Kingdom.

Passage History:

X/C2 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; C = CaCO-2 cells¹

Lot: 70025944² Manufacturing Date: 21JUN2019

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 (~ 430 nucleotides)	≥ 98% identity with HAstV3, Oxford (GenBank: MK059951.1)	99.8% identity with HAstV3, Oxford (GenBank: MK059951.1)
Titer by TCID₅₀ Assay in CaCO-2 cells with IFA Readout¹,₃,₄,₅	Report results	8.9 × 10 ⁶ TCID ₅₀ per mL
Amplification of HAstV3 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 ⁶ 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/

Heather Couch 30 SEP 2019

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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BEI Resources

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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, Clone 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

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.