

## **Certificate of Analysis for NR-51388**

## **Human Astrovirus Type 1, Oxford**

Catalog No. NR-51388

**Product Description:** Human astrovirus type 1 (HAstV1), Oxford was isolated from the stool of a human with acute gastroenteritis in Oxford, United Kingdom.

Passage History: X/C5 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown;

 $C = CaCO-2 cells^1$ 

Lot<sup>2,3</sup>: 70019565 Manufacturing Date: 26MAR2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using CaCO-2 Cells <sup>1</sup>	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 420 nucleotides)	≥ 98% identity with HAstV1, Oxford (GenBank: MK059949.1)	99.8% identity with HAstV1, Oxford (GenBank: MK059949.1)
Titer by TCID <sub>50</sub> Assay <sup>5,6</sup> in CaCO-2 cells <sup>1</sup> with IFA Readout <sup>4</sup>	Report results	$2.8 \times 10^7  \text{TCID}_{50}  \text{per mL}$
Amplification of HAstV1 Sequence by RT-PCR	~ 450 base pair amplicon	~ 450 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth <sup>7</sup> , 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 <sub>2</sub>	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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**BEI Resources** 

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<sup>&</sup>lt;sup>2</sup>The second viral passage at BEI Resources was performed by polyethylenimine (Polyplus-transfection® SA jetPEI® 101-10)-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

<sup>&</sup>lt;sup>3</sup>Grown 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<sup>®</sup> 30-2003) supplemented with 1 μg/mL trypsin type IX-S for 1 day at 37°C with 5% CO<sub>2</sub>. 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.

<sup>&</sup>lt;sup>4</sup>Using Monoclonal Astrovirus Type 1-5, Clone J12H (ThermoFisher Scientific MA5-18174)

<sup>&</sup>lt;sup>5</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>6</sup>Assay plates were incubated 6 days at 37°C and 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>7</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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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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