

Certificate of Analysis for NR-51392

Human Astrovirus Type 5, Oxford

Catalog No. NR-51392

Product Description:

Human astrovirus type 5 (HAstV5), Oxford was isolated from the stool of a human with acute gastroenteritis in Oxford, United Kingdom. HAstV5, Oxford was grown in human colon adenocarcinoma cells (CaCO-2; ATCC® HTB-37 $^{\text{TM}}$) in Eagle's Minimum Essential Medium (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.

Passage History:

X(?)/C(3) (Prior to deposit /BEI Resources); X = Unknown; C = CaCO-2 cells

Lot: 70029443 Manufacturing Date: 07NOV2019

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in CaCO-2 Cells	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay¹	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~420 nucleotides)	≥ 98% identity with HAstV5, Oxford (GenBank: MK059953.1)	100% identity with HAstV5, Oxford (GenBank: MK059953.1)
Titer by TCID ₅₀ Assay in CaCO-2 cells with IFA Readout ^{1,2}	Report results	2.8 x 10 ⁸ TCID ₅₀ per mL after 6 days at 37°C and 5% CO ₂
Amplification of HAstV5 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 ³	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 ₂	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

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

/Heather Couch/ Heather Couch

24 FEB 2020

Program Manager or designee, ATCC Federal Solutions

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²The 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.
³Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.