b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50895

Murine Norovirus 1, CW3, Infectious Clone

Catalog No. NR-50895

Product Description: Murine norovirus 1 (MNV-1), clone CW3 was isolated in 2002 from brain tissue of STAT$^{-/-}$ mice infected with MNV-1 by the oral route. It was plaque purified three times prior to deposit to BEI Resources.

Passage History: B2/R2 (Prior to deposit at BEI Resources/BEI Resources); B = Mouse BV2 cells; R = Mouse RAW 264.7 cells[1]

Lot[1,2]: 70016130 **Manufacturing Date: 02JUL2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in RAW 264.7 cells[1]	Cell rounding and detachment	Cell rounding and detachment
Whole Genome Sequencing (~ 7350 nucleotides)	≥ 98% identity with MNV-1, Clone CW3 (GenBank: EF014462.1)	100% identity with MNV-1, Clone CW3 (GenBank: EF014462.1)
Titer by $TCID_{50}$ Assay[3,4] in RAW 264.7 cells[1] by Cytopathic Effect	Report results	8.9×10^5 $TCID_{50}$ per mL
Amplification of MNV-1 Sequence by RT-PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[5], 37°C and 26°C, aerobic	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
Blood agar, 37°C, aerobic	No growth	No growth
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

[1]*Mus musculus* macrophage cells (RAW 264.7; ATCC® TIB-71™)
[2]Grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 mg per L glucose, 1 mM sodium pyruvate, and 1500 mg per L sodium bicarbonate (ATCC® 30-2002) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 5 days at 37°C with 5% CO_2
[3]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[4]Assay plates were incubated 6 days at 37°C and 5% CO_2
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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