b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-42515

Mammalian Rubulavirus 5, 21005-2WR (Tissue Culture Adapted) (formerly Parainfluenza Virus 5)

Catalog No. NR-42515

This reagent is the property of the U. S. Government.

Product Description:

Mammalian rubulavirus 5 was first isolated from monkey kidney cell cultures by Dr. Robert Hull in 1954.

Passage History:

RhMK(10)LLC-MK2(6)GMK(1)CE(12)/LLC-MK2(5) (Prior to deposit at BEI Resources/BEI Resources); RhMK= Rhesus Monkey kidney cells; GMK = African green monkey kidney cells; CE = Chicken embryo; LLC-MK2 = *Macaca mulatta* (Rhesus Monkey) kidney cells[1]

Lot: 70026761[1-3] **Manufacturing Date: 12JUL2019**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in LLC-MK2 cells	Cell rounding and sloughing	Cell rounding and sloughing
Sequencing of Species-Specific Region (~ 840 nucleotides)	Consistent with mammalian rubulavirus	97.6% identity with mammalian rubulavirus 5 (GenBank: KY685075.1)[4]
Titer by $TCID_{50}$ Assay in LLC-MK2 Cells by Hemagglutination Assay[1,5,6,7]	Report results	1.6×10^6 $TCID_{50}$ per mL
Amplification of Mammalian Rubulavirus 5 Sequence by RT-PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[8]	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] *Macaca mulatta* kidney epithelial cells (LLC-MK2, ATCC® CCL-7.1™)

[2] Derived from NIAID Catalog No. V-322-011-000

[3] Lot 70026761 of NR-42515 was produced by infecting LLC-MK2 cells with BEI Resources NRS-42515 lot 61389532 and incubating in Dulbecco's Modified Eagle's Minimum Essential Medium (ATCC® 30-2002) supplemented with 4 µg/mL trypsin and 10% fetal bovine serum (ATCC® 30-2020) for 4 days at 37°C with 5% CO_2.

[4] Sequence information for mammalian rubulavirus 5, 21005-2WR is not available in the NCBI database; nucleotide sequence obtained for NR-42515 lot 70026761 has 100% identity to the sequence of the previous lot of NR-42515 and 97.6% identity to mammalian rubulavirus 5 (GenBank: KY685075.1). Although the identity is less than 98%, the sequence obtained matched only parainfluenza virus 5, simian virus 5, and mammalian rubulavirus 5 when BLAST was performed.

[5] 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.

[6] Assay plates were incubated 7 days at 37°C and 5% CO_2.

[7] HA was performed with 0.5% turkey red blood cells.

[8] Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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/Heather Couch/
Heather Couch 25 OCT 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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