

# **Certificate of Analysis for NR-51430**

## Enterovirus D68, Fermon

#### Catalog No. NR-51430

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## **Product Description:**

Enterovirus D68 (EV-D68), Fermon was isolated from a nasopharyngeal swab from a hospitalized pediatric patient with pneumonia in California, USA in 1962. NR-51430 lot 70028609 was produced by infecting human lung fibroblasts (MRC- 5; ATCC<sup>®</sup> CCL-171™) with deposited material (V-067-002-020) and incubating in Eagle's MEM (ATCC<sup>®</sup> 30-2003) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020) for 3 days at 33°C with 5% CO<sub>2</sub>.

## Passage History:

MK(6)HFD(7)HELF(2)pAGMK(4)/MRC-5(3) (Prior to deposit with BEI Resources/BEI Resources); MK = Monkey kidney cells; HFD = Human fetal diploid cells; HELF = Human embryonic lung fibroblasts; pAGMK = Primary African Green monkey kidney cells; MRC-5 = Human lung fibroblasts

Lot: 70028609 Manufacturing Date: 18OCT2019

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MRC-5 cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (810 nucleotides)	≥ 98% identity with EV-D68, Fermon (GenBank: AY426531.1)	99.9% identity with EV-D68, Fermon (GenBank: AY426531.1)
Titer by TCID <sub>50</sub> Assay in MRC-5 cells by Cytopathic Effect <sup>1</sup>	Report results	1.6 × 10 <sup>6</sup> TCID <sub>50</sub> per mL in 6 days at 33°C with 5% CO <sub>2</sub>
Amplification of EV-D68 Sequence by RT-PCR	~ 870 base pair amplicon	~ 870 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup>	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 <sub>2</sub>	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

<sup>&</sup>lt;sup>1</sup>The 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.

/Heather Couch/

Heather Couch 13 FEB 2020

Program Manager or designee, ATCC Federal Solutions

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