

## **Certificate of Analysis for NR-51**

## Vaccinia Virus, Lister (Elstree)

Catalog No. NR-51

(Derived from ATCC® VR-1549™)

**Product Description:** Vaccinia virus (VACV), strain Lister (Elstree) was deposited to ATCC® by Dr. James H. Nakano at the Centers for Disease Control in 1978. The Lister (Elstree) strain was widely used during the World Health Organization program on the eradication of smallpox.

**Passage History:** LXV3/V3/V1 (Prior to deposit to ATCC/ATCC/BEI Resources); LX= Unknown passages as Lister vaccine; V = Vero cells<sup>1</sup>

Lot<sup>2</sup>: 70018915 Manufacturing Date: 15OCT2018

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells <sup>1</sup>	Rounding and detachment	Rounding and detachment
Sequencing of Species-Specific Region (~ 970 nucleotides)	≥ 98% identity with VACV, Lister (Elstree) (GenBank: AY678276.1)	99.9% identity with VACV, Lister (Elstree) (GenBank: AY678276.1)
Titer by TCID₅₀ Assay³,⁴ in Vero cells¹ by Cytopathic Effect	Report results	$8.9 \times 10^7 \text{ TCID}_{50} \text{ per mL}$
Amplification of VACV Sequence by PCR	~ 1100 base pair amplicon	~ 1100 base pair amplicon
Sterility (23-day incubation) Harpo's HTYE broth <sup>5</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 (18-day incubation at 37°C)  DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

<sup>&</sup>lt;sup>1</sup>Cercopithecus aethiops kidney epithelial cells (Vero cells: ATCC® CCL-81™)

## /Heather Couch/

Heather Couch 21 JAN 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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<sup>&</sup>lt;sup>2</sup>Lot 70018915 of NR-51 was produced by infecting Vero cells¹ with BEI Resources NRS-51 lot 3580747 and incubating 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 (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 4 days at 37°C with 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</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>4</sup> The culture was incubated for 5 days at 37°C and 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>5</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.