

Certificate of Analysis for NR-727

Vaccinia Virus, Modified Vaccinia Ankara (MVA), Purified From BHK-21 Cells

Catalog No. NR-727

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

Product Description: NR-727 was prepared by inoculation of hamster kidney cells¹ with VACV, MVA². The virus was purified from a crude cell lysate and supernatant by centrifugation through a 36% sucrose cushion.

Lot³: 62111431 Manufacturing Date: 15OCT2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in BHK-21 Cells ¹	Cell rounding and cell lysis	Cell rounding and cell lysis
PCR Amplification of Strain-Specific Sequence (985 nucleotides)	Vaccinia virus, MVA	Vaccinia virus, MVA
Titer by Plaque Assay ⁴ in VERO C1008 (E6) Cells ⁵	Report results	1.9 × 10 ⁷ pfu per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic 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) DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

¹BHK-21 cells: ATCC[®] CCL-10™

Date: 24 FEB 2014

Signature:

Title:

Technical Manager, BEI Authentication or designee

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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²NR-727 was prepared by two passages of vaccinia virus, MVA (BEI Resources NR-726, lot 4225252) in BHK-21 cells followed by purification through a 36% sucrose cushion. BEI Resources NR-726, lot 4225252 was prepared by one passage in BHK-21 cells from BEI Resources NR-1, lot 3564849. The inoculum for BEI Resources NR-1, lot 3564849 was prepared in chicken embryo fibroblast (CEF) cells and provided by the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

³Grown in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 10% fetal bovine serum (ATCC® 30-2020™) for 3 days at 37°C and 5% CO₂

⁴72 hours at 37°C and 5% CO₂ with media overlay and crystal violet staining

⁵VERO C1008 (E6) cells: ATCC[®] CRL-1586™; also available as BEI Resources NR-596

⁶Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798