

Certificate of Analysis for NR-726

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

Catalog No. NR-726

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

Product Description: Cell lysate and supernatant from hamster kidney cells (BHK-21) cells infected with vaccinia virus, MVA.²

Lot³: 4225252 Manufacturing Date: 18APR2005

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	Vaccinia virus, MVA	Vaccinia virus, MVA
Titer by TCID ₅₀ Assay ^{4,5} in VERO C1008 (E6) Cells ⁶	Report results	2.8 X 10 ⁷ TCID ₅₀ /mL
Titer by Plaque Assay ⁷ in VERO C1008 (E6) Cells ⁶	Report results	3.9 X 10 ⁷ pfu/mL
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 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 extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹BHK-21 cells: ATCC[®] CCL-10™.

Date: 29 JAN 2007 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication

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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Biodefense and Emerging Infections Research Resources Repository P.O. Box 4137

Manassas, VA 20108-4137 USA www.beiresources.org

E-mail: contact@beiresources.org

800-359-7370

Fax: 703-365-2898

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²Vaccinia virus, MVA: 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 Minimum Essential Medium containing Earle's salts and non-essential amino acids (GIBCO[®] 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex 14-471F), 2 mM L-glutamine (GIBCO[®] 25030-081), and 1 mM sodium pyruvate (GIBCO[®] 11360-070) for 4 days at 37°C and 5% CO₂.

⁴The 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.

⁵4 days at 37°C and 5% CO₂ with media overlay.

⁶VERO C1008 (E6) cells: ATCC[®] CRL-1586[™]; also available as BEI Resources NR-596.

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

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