Certificate of Analysis for NR-59630

First World Health Organization International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing

Catalog No. NR-59630

Product Description:

NR-59630 consists of a panel of seven purified viruses intended to be used together as reference standards for method verification/qualification and validation by high-throughput sequencing/next generation sequencing (HTS/NGS) technologies. Viruses should be used directly, without amplification or propagation.

The viruses were prepared by U.S. Food and Drug Administration/Center for Biologics Evaluation and Research (FDA/CBER) under contract number 75F40120P00620 and 75F40120P00690 with ATCC.

Lot: 70068950 **Assembly Date: 09NOV2023**

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

Table 1: Kit Components

COMPONENT NUMBER	VIRUS	STRAIN (SOURCE)	CELL LINE USED FOR PROPAGATION (ATCC CATALOG NUMBER)	LOT NUMBER	VIALING DATE
CBER-FSCUST-90™	Purified betacoronavirus (hCoV)	OC43 (ATCC® VR-1558™)	HRT-18G cells (ATCC® CRL-11663™)	70047896	23NOV2021
CBER-FSCUST-91™	Purified porcine circovirus (PCV)	Type 1 (persistently infected cell line ATCC® CCL-33™)	PK(15) cells (ATCC® CCL-33™)	70048776	31MAY2022
CBER-FSCUST-92™	Purified mammalian orthoreovirus (REO)	Lang (ATCC® VR-230™)	LLC-MK2 derivative cells (ATCC® CCL-7.1™)	70048775	24MAY2022
CBER-FSCUST-93™	Purified feline leukemia virus (FELV)	KT-FeLV-UCD-1 (persistently infected cell line ATCC® CRL-8012™)	FL74-UCD-1 cells (ATCC® CRL-8012™)	70048774	08JUN2022
CBER-FSCUST-94™	Purified Epstein-Barr virus (human herpes virus 4) (EBV/HHV4)	B95-8 (persistently infected cell line ATCC® CRL-1612™)	B95-8 Leukocyte Marmoset culture (ATCC® CRL-1612™)	70048773	21APR2022
CBER-FSCUST-95™	Purified respiratory syncytial virus (RSV)	A2 (ATCC® VR-1540™)	HEp-2 cells (ATCC® CCL-23™)	70048772	16NOV2021
CBER-FSCUST-96™	Purified minute virus of mice (MVM)	Prototype (p) (ATCC® VR-1346™)	A9 cells (ATCC® CCL-1.4™)	70044330	08SEP2021

Table 2: hCoV, OC43 (CBER-FSCUST-90™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1×10^6 $TCID_{50}$/mL	1.58×10^7 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8]	≥ 1×10^{10} genome copies/mL	2.64×10^{10} genome copies/mL

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Purified Betacoronavirus (hCoV), OC43 (ATCC® VR-1558™) was grown in HRT-18G cells (ATCC® CRL-11663™) at 33°C with humidity and 5% CO_2.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that was harvested on different dates. Dates of harvest for bulk materials were 20JUL2021 and 20SEP2021. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2022 shows that the material maintains these characteristics when stored at -80°C.
[5]8 days in HRT-18G cells (ATCC® CRL-11663™) at 33°C with 5% CO_2, as determined by CPE.
[6]ddPCR data was obtained post-vial from 18 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was replicase polyprotein Orf1ab (GenBank: NC_006213.1).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Host cell genome copy number is 1.64 x 10^4 genome copies/mL.

Table 3: PCV Type 1 (CBER-FSCUST-91™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1 × 10^6 $TCID_{50}$/mL	2.32 × 10^6 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8]	≥ 1 × 10^{10} genome copies/mL	8.07× 10^9 genome copies/mL
Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Purified porcine circovirus, type 1 was grown in PK(15) cells (ATCC® CCL-33™) at 37°C with humidity and 5% CO_2. The virus stock contains porcine endogenous retrovirus (PERV; GenBank: AF038600.1), which is known to be present in the PK(15) cells [Pol. J. Microbiol. (2012), 61: 211-215. PubMed: 29334069]. Genome copy number for PERV is 1.08 x 10^9 genome copies/mL.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that was harvested on different dates. Dates of harvest for bulk materials were 03DEC2021, 19JAN2022, 02FEB2022 and 02MAY2022. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2023 shows that the material maintains these characteristics when stored at -80°C.
[5]16 days in ST cells (ATCC® CRL-1746™) at 37°C with 5% CO_2, as determined by PCR.
[6]ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was replication associated protein (GenBank: NC_001792.2).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Host cell genome copy number is 9.81 x 10^3 genome copies/mL.

Table 4: REO, Lang (CBER-FSCUST-92™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1 × 10^6 $TCID_{50}$/mL	8.89 × 10^8 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8,9]	≥ 1 × 10^{10} genome copies/mL	1.50 × 10^{10} genome copies/mL
Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Purified mammalian reovirus (MRV), Lang (ATCC® VR-230™) was grown in LLC-MK2 derivative cells (ATCC® CCL-7.1™) at 37°C with humidity and 5% CO_2.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that was harvested on different dates. Dates of harvest for bulk materials were 27APR2021 and 02SEP2021. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2023 shows that the material maintains these characteristics when stored at -80°C.
[5]8 days in LLC-MK2 derivative cells (ATCC® CCL-7.1™) at 37°C with 5% CO_2, as determined by CPE.
[6]ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was core protein L3 (GenBank: AF129820.1).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Residual bovine bosavirus sequences were detected by NGS analysis.
[9]Host cell genome copy number is below the level of detection.

Table 5: FELV, KT-FeLV-UCD-1 (CBER-FSCUST-93™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1 × 10^6 $TCID_{50}$/mL	1.99 × 10^7 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8]	≥ 1 × 10^{10} genome copies/mL	4.01 × 10^{10} genome copies/mL
Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Purified feline leukemia virus, Theilen was grown in FL74-UCD-1 cells (ATCC® CRL-8012™) at 37°C with humidity and 5% CO_2.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that was harvested on different dates. Dates of harvest for bulk materials were 10AUG2021, 13AUG2021, 07SEP2021 and 10SEP2021. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2023 shows that the material maintains these characteristics when stored at -80°C.
[5]7 days in CRFK cells (ATCC® CCL-94™) at 37°C with 5% CO_2, as determined by PCR.
[6]ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was envelope polyprotein (GenBank: MT129531.1).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Host cell genome copy number is 2.05 x 10^5 genome copies/mL.

Table 6: EBV/HHV4, B95-8 (CBER-FSCUST-94™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1 × 10^6 $TCID_{50}$/mL	1.12 × 10^6 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8]	≥ 1 × 10^{10} genome copies/mL	2.82 × 10^7 genome copies/mL[9]
Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Purified Epstein-Barr virus (human herpes virus 4), B95-8 was isolated from B95-8 Leukocyte Marmoset culture (ATCC® CRL-1612™) grown at 37°C with humidity with 5% CO_2. The virus stock contains SMRV (GenBank: NC_001514.1), which is known to be present in the B95-8 cell line [Virology. (1995), 209: 374-383. PubMed: 7778272]. Genome copy number for SMRV is 1.80 x 10^{10} genome copies/mL.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that was harvested on different dates. Dates of harvest for bulk materials were 21JUN2021, 29JUN2021, 30JUL2021, 02AUG2021, 06AUG2021 and 23AUG2021. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2023 shows that the material maintains these characteristics when stored at -80°C.
[5]46 days in IRR-MRC-5 Irradiated Fibroblast Human cells (ATCC® 55-X™) at 37°C with 5% CO_2, as determined by transformation.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

[6]ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was EBER1 noncoding RNA (GenBank: V01555.2).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Host cell genome copy number is 9.59 x 10^4 genome copies/mL.
[9]The genome copy number for HHV-4, strain B95-8 is below the current specifications but does not negatively impact the final product.

Table 7: RSV, A2 (CBER-FSCUST-95™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1 × 10^6 $TCID_{50}$/mL	5.00 × 10^6 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8,9]	≥ 1 × 10^{10} genome copies/mL	5.53 × 10^{10} genome copies/mL
Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Purified respiratory syncytial virus, A2 (ATCC® VR-1540™) was grown in HEp-2 cells (ATCC® CCL-23™) at 37°C with humidity and 5% CO_2.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that was harvested on different dates. Dates of harvest for bulk materials were 02AUG2021, 04AUG2021, 27SEP2021, 04OCT2021 and 13OCT2021. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2022 shows that the material maintains these characteristics when stored at -80°C.
[5]7 days in HEp-2 cells (ATCC® CCL-23™) at 37°C with 5% CO_2, as determined by CPE and DFA with LIGHT DIAGNOSTICS™ Respiratory Syncytial Virus FITC Reagent (Millipore Sigma 5022).
[6]ddPCR data was obtained post-vial from 11 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was N protein (GenBank: JF920069.1).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Human papillomavirus sequences detected by NGS analysis due to Hep-2 cells.
[9]Host cell genome copy number is 1.54 x 10^6 genome copies/mL.

Table 8: MVM Prototype (p) (CBER-FSCUST-96™)[1,2,3]

Test / Method	Specification	Result
Titer (Post-vial)[4,5]	≥ 1 × 10^6 $TCID_{50}$/mL	1.58 × 10^7 $TCID_{50}$/mL
Genome Copy Number by ddPCR (Post-vial)[4,6,7,8]	≥ 1 × 10^{10} genome copies/mL	1.18 × 10^{10} genome copies/mL
Test for Mycoplasma Contamination DNA detection by PCR of test article nucleic acid [Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)]	None detected	None detected
Sterility Test (BacT/ALERT 3D) iAST bottle (aerobic) at 32°C, 14-day incubation iNST bottle (anaerobic) at 32°C, 14-day incubation	 No growth No growth	 No growth No growth

[1]Minute virus of mice, Prototype (p) (ATCC® VR-1346™) was grown in A9 cells (ATCC® CCL-1.4™) at 37°C with humidity and 5% CO_2.
[2]Preparation was vialed in 10 mM Tris-HCl, 135 mM NaCl, 0.5% BSA and 5% trehalose and may contain residual cellular nucleic acids.
[3]The product was vialed from pooled bulk material that were harvested on different dates. Dates of harvest for bulk materials were 12APR2021, 12MAY2021, 21MAY2021, 08JUN2021. All bulks were frozen following harvest.
[4]Stability testing of the genome copy number and titer completed in 2022 shows that the material maintains these characteristics when stored at -80°C.
[5]8 days in A9 cells (ATCC® CCL-1.4™) at 37°C with 5% CO_2, as determined by CPE and IFA with MVMVP21-S antibody at 1:200 dilution.
[6]ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System; gene assayed was nonstructural protein NS-1 (GenBank: J02275.1).
[7]ddPCR samples contain virus genomes and may have residual mRNAs.
[8]Host cell genome copy number is 3.50 x 10^5 genome copies/mL.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Certificate of Analysis for NR-59630

/Sonia Bjorum Brower/
Sonia Bjorum Brower 22 MAY 2024
Technical 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 by ATCC® and the contributor 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.

Support Provided by NIAID

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898