

Certificate of Analysis for NR-48812

Middle East Respiratory Syndrome Coronavirus, Recombinant Infectious Clone with T1015N Tissue Culture Adaptation Mutation (icMERS-CoV-T1015N)

Catalog No. NR-48812

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with Middle East respiratory syndrome coronavirus, recombinant infectious clone with T1015N tissue culture adaptation mutation (icMERS-CoV-T1015N)

Passage History: Submission laboratory: Vero (1); BEI Resources: Vero (2)

Lot²: 63541998 Manufacturing Date: 25JUN2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Refractile cell rounding and detachment	Refractile cell rounding and detachment
Sequencing of Strain-Specific Region (728 nucleotides)	Consistent with MERS-CoV T1015N mutation confirmed	99% identity with MERS-CoV, EMC/2012 (GenBank: JX869059) T1015N mutation confirmed
Titer by TCID ₅₀ Assay in Vero Cells ^{1,3,4}	Report results	$2.8 \times 10^7 TCID_{50} per mL$
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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

¹Vero cells: ATCC[®] CCL-81™

Date: 06 OCT 2016

Signature:

BEI Resources Authentication

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²Grown in Dulbecco's Modified Eagle's Medium (ATCC[®] 30-2002) supplemented with 2% fetal bovine serum (ATCC[®] 30-2020) for 2 days at 37°C and 5% CO₂

³TCID₅₀ (50% tissue culture infectious dose): The TCID₅₀ is the 50% infectious endpoint in tissue 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. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer of a virus preparation.

⁴8 days at 37°C and 5% CO₂

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