b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-43001

## Recombinant Murine Coronavirus, icA59-ns2dm

## Catalog No. NR-43001

**Product Description:** Cell lysate and supernatant from *mus musculus* liver epithelial cells[1] infected with recombinant murine coronavirus, icA59-ns2dm

**Lot[2]: 61556176** **Manufacturing Date: 15MAY2013**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Identification by Infectivity in NCTC clone 1469 Cells[1]** | Cell enlargement and detachment | Cell enlargement and detachment |
| **Sequencing of Species-Specific Region (659 nucleotides)** | Consistent with murine coronavirus, icA59-ns2dm | 100% identity with murine coronavirus, icA59-ns2dm (GenBank: KF268339)[3,4] |
| **Titer by $TCID_{50}$ Assay[5,6] in NCTC clone 1469 Cells[1]** | Report results | $2.3 \times 10^7$ $TCID_{50}$ per mL |
| **Sterility (21-day incubation)** | | |
| Harpo's HTYE broth[7], 37°C and 26°C, aerobic | No growth | No growth |
| Trypticase soy broth, 37°C and 26°C, aerobic | No growth | No growth |
| Sabouraud broth, 37°C and 26°C, aerobic | No growth | No growth |
| Sheep blood agar, 37°C, aerobic | No growth | No growth |
| Sheep blood agar, 37°C, anaerobic | No growth | No growth |
| Thioglycollate broth, 37°C, anaerobic | No growth | No growth |
| 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) | None detected | None detected |
| DNA Detection by PCR of Test Article nucleic acid | None detected | None detected |

[1]NCTC clone 1469 cells (ATCC® CCL-9.1™)
[2]Grown in Dulbecco's Modified Eagle Medium (ATCC® 30-2002™) supplemented with 10% fetal bovine serum (ATCC® 30-2020™) for 1 day at 37°C and 5% $CO_2$
[3]Note that the nomenclature used for this virus in the NCBI database differs from that used here.
[4]Sequence analysis at BEI Resources confirmed the presence of the H46A and H126R mutations described in Roth-Cross, J. K., et al. "Organ-Specific Attenuation of Murine Hepatitis Virus Strain A59 by Replacement of Catalytic Residues in the Putative Viral Cyclic Phosphodiesterase ns2." J. Virol. 83 (2009): 3743-3753. PubMed: 19176619.
[5]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% ($LD_{50}$) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[6]9 days at 37°C and 5% $CO_2$
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 21 OCT 2013 **Signature:** Michael R. Gyrla

**Title:** Technical Manager, BEI Authentication or designee

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