

Certificate of Analysis for NR-43000

Recombinant Murine Coronavirus, icA59

Catalog No. NR-43000

Product Description: Clarified cell lysate and supernatant from *mus musculus* liver epithelial cells¹ infected with recombinant murine coronavirus, icA59

Lot²: 61556175 Manufacturing Date: 03JUL2013

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 (657 nucleotides)	Consistent with murine coronavirus, icA59	100% identity with murine coronavirus, icA59 (GenBank: KF268337) ³
Titer by TCID ₅₀ Assay ^{4,5} in NCTC clone 1469 Cells ¹	Report results	1.6 × 10 ⁷ TCID ₅₀ 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

¹NCTC clone 1469 cells (ATCC[®] CCL-9.1™)

Date: 21 OCT 2013

Signature.

Title: Technical Manager, BÉI Authentication or designee

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²Grown in Dulbecco's Modified Eagle Medium (ATCC® 30-2002™) supplemented with 2% horse serum (ATCC® 30-2040™) for 2 days at 37°C and 5% CO₂

Note that the nomenclature used for this virus in the NCBI database differs from that used here.

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

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