

Certificate of Analysis for NR-43284

Avian Coronavirus, Massachusetts (formerly Avian Infectious Bronchitis Virus)

Catalog No. NR-43284

Derived from BEI Resources NR-444

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with the Massachusetts strain of avian coronavirus

Lot²: 61617359 Manufacturing Date: 05DEC2013

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (430 nucleotides)	Consistent with avian coronavirus, Massachusetts	99% identity with avian coronavirus, Massachusetts (GenBank: GQ504724)
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹ Using IFA Readout ³	Report results	2.8 × 10 ⁷ CEID ₅₀ per mL
Functional Activity by RT-PCR Assay	~ 430 bp amplicon	~ 430 bp amplicon
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 Test Article nucleic acid	None detected None detected	None detected None detected

¹9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 31 MAR 2014

Signature:

Title:

Technical Manager, BEI Authentication or designee

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

Fax: 703-365-2898

²Grown in the allantoic cavity of embryonated chicken eggs¹ for 3 days at 37°C in a humidified chamber

³Using BEI Resources NR-2515, Polyclonal Anti-Avian Infectious Bronchitis Virus, Massachusetts (antiserum, Guinea Pig)

⁴The Chicken Embryo Infectious Dose 50% (CEID₅₀) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the inoculated embryonated chicken eggs, 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 CEID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁵3 days at 37°C in a humidified chamber.

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