

Certificate of Analysis for NR-2757

Influenza A Virus, A/duck/Germany/1215/73 (H2N3)

Catalog No. NR-2757

(Derived from ATCC® VR-1328™)

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A virus, A/duck/Germany/1215/73 (H2N3).

Lot²: 7677041 Manufacturing Date: 15SEP2006

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination assay	Active	Active
Sequencing of Species-Specific Region	Influenza A virus	Influenza A virus
Titer by CEID ₅₀ Assay ^{3,4} in Embryonated Chicken Eggs ¹	Report results	2.8 X 10 ⁷ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA ⁵	~ 1030 bp amplicon	~ 1030 bp amplicon
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 extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹10-to-11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, PA.

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Date: 10 OCT 2007 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²NR-2757 was produced by growth of ATCC[®] VR-1328™ (Lot 214335) in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber without CO₂.

 $^{^3}$ The Chicken Embryo Infectious Dose 50% (CEID $_{50}$) 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% (LD $_{50}$) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the CEID $_{50}$ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁴48 hours at 35°C in a humidified chamber without CO₂.

⁵BM-M1 and BM-M-1027R primers; Obenauer, J. C., et al. "Large-Scale Sequence Analysis of Avian Influenza Isolates." <u>Science</u> 311 (2006): 1576–1580. PubMed: 16439620.

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