

**Kilbourne F164: A/Dunedin/6/1983 (HA, NA) x A/Puerto Rico/8/1934 (H1N1),
Reassortant X-81a**

Catalog No. NR-3471

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/Dunedin/6/1983 (HA, NA) x A/Puerto Rico/8/1934 (H1N1)

Lot^{2,3}: 61327891

Manufacturing Date: 31OCT2012

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs¹ Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (402 nucleotides) Matrix (906 nucleotides)	Consistent with A/New Zealand/7/1983 (H1N1) ⁴ Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/New Zealand/7/1983 (H1N1) (GenBank: CY020189) ⁴ 100% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY105897)
Titer by CEID₅₀ Assay^{5,6} in Embryonated Chicken Eggs¹	Report results	8.9 × 10 ⁹ CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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

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

²Derived from NIAID Catalog No. V-331-0E4154

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber

⁴There is no sequence information for influenza A virus, A/Dunedin/6/1983 (H1N1) in the NCBI database or any of the various influenza databases. The hemagglutinin gene sequence obtained for NR-3471 is 99% identical to the published sequence of influenza A virus, A/New Zealand/7/1983 (H1N1), which was originally isolated at Dunedin Hospital during a major influenza outbreak in 1983. The NR-3471 HA sequence is also 99% identical to the HA gene sequence of influenza A/CHR/157/1983 (H1N1), which was also isolated during the 1983 New Zealand influenza outbreak.

⁵The 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.

⁶2 days at 35°C in a humidified chamber

⁷Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Certificate of Analysis for NR-3471

Date: 22 JAN 2013

Signature: *Michael R. Gynther*

Title: Technical Manager, BEI Authentication or designee

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