

**Kilbourne F78: A/Beijing/32/1992 (HA, NA) x A/Puerto Rico/8/1934 (H3N2),
Reassortant X-117**

Catalog No. NR-3529

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/Beijing/32/1992 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)

Lot^{2,3}: 60341182

Manufacturing Date: 04NOV2011

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 (614 nucleotides) Matrix (885 nucleotides)	Consistent with A/Beijing/32/1992 (H3N2) Consistent with A/Puerto Rico/8/1934 (H1N1)	100% identity with A/Beijing/32/1992 (H3N2) (GenBank: CY033606) ⁴ 100% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY045765) ⁵
Titer by CEID₅₀ Assay^{6,7} in Embryonated Chicken Eggs¹	Report results	5.0 × 10 ⁹ CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 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

¹10- to 11-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

²Derived from NIAID Catalog No. V-331-0E5120

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

⁴The sequence of the Hemagglutinin gene from Reassortant X-117 has also been deposited to the NCBI database (GenBank CY036807) and is identical to that of the parental A/Beijing/32/1989 virus.

⁵The sequence of the Matrix gene from Reassortant X-117 has also been deposited to the NCBI database (GenBank CY036808) and is identical to that of the parental A/Puerto Rico/8/1934 virus.

⁶The 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.

⁷2 days at 35°C in a humidified chamber

⁸Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798

Certificate of Analysis for NR-3529

Date: 09 MAY 2012

Signature: *Dorothy C. Young*

Title: Technical Manager, BEI Authentication or designee

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