Certificate of Analysis for NR-19867

Monoclonal Anti-Influenza Virus H1 Hemagglutinin (HA), A/California/04/2009 (H1N1)pdm09, Clone S-OIV-12F3 (produced *in vitro*)

Catalog No. NR-19867

This reagent is the property of the U.S. Government.

Product Description: Mouse monoclonal antibody (clone S-OIV-12F3) prepared against the H1 hemagglutinin (HA) protein of the A/California/04/2009 (H1N1)pdm09 strain of influenza virus was purified from clone S-OIV-12F3 hybridoma supernatant by protein G affinity chromatography.

Lot: 59476517 **Manufacturing Date: 09NOV2010**

TEST	SPECIFICATIONS	RESULTS
Antibody Class Determination	Report results	IgG2aκ
Experion Pro260 Analysis	Correct molecular weight (MW) for heavy and light chains Report results	Correct MW for heavy and light chains (Figure 1) 96.9% pure
Concentration by Spectrophotometer at OD_{280}	Report results	1.0 mg/mL
Functional Activity Indirect Immunofluorescence Assay A/California/04/2009 (H1N1)pdm09[1] A/swine/Iowa/15/1930 (H1N1)[2] ELISA Indirect[3] Sandwich[4]	 Fluorescence observed No fluorescence observed Report results Report results	 Weak fluorescence observed (Figure 2) No fluorescence observed Not reactive Not tested
Sterility	0.22 µm filter-sterilized	0.22 µm filter-sterilized

[1]MDCK cells (ATCC® CCL-34™) were infected with influenza virus A/California/04/2009 (H1N1)pdm09 (BEI Resources NR-13658) at an MOI of 0.1 and stained 3 days later with a 1:100 dilution of NR-19867 and FITC-conjugated goat anti-mouse IgG F(ab')2 fragment (Millipore 5008).

[2]MDCK cells (ATCC® CCL-34™) were infected with influenza virus A/swine/Iowa/15/1930 (H1N1) (ATCC® VR-1683™) at an MOI of 0.1 and stained 3 days later with a 1:100 dilution of NR-19867 and FITC-conjugated goat anti-mouse IgG F(ab')2 fragment (Millipore 5008).

[3]Wells were coated with 20 ng of recombinant H1 HA from influenza virus A/California/04/2009 (H1N1)pdm09 (BEI Resources NR-15749), blocked and incubated with a 1:100 dilution of NR-19867 followed by biotin-conjugated goat anti-mouse IgG + IgM (H & L) (Rockland Immunochemicals 610-106-115), peroxidase-conjugated streptavidin (Rockland Immunochemicals S000-03) and TMB ELISA peroxidase substrate (Rockland Immunochemicals TMBE-1000). Absorbance was read at 450 – 650 nm. NR-15479 was readily detected in this ELISA format using either monoclonal anti-influenza virus H1 HA, A/California/04/2009 (H1N1)pdm09, clone S-OIV-3B2 (BEI Resources NR-19864) or monoclonal anti-influenza virus H1 HA, A/California/06/2009 (H1N1)pdm09, clone 2F3 (Immune Technology IT-003-001M5).

[4]In combination with NR-19864 and NR-19866, NR-19867 has been used in a sandwich ELISA to distinguish influenza A (H1N1)pdm09 viruses from other swine-origin H1 viruses as well as human seasonal H1N1 and H3N2 viruses (Shao, H., et al. "A Monoclonal Antibody-Based ELISA for Differential Diagnosis of 2009 Pandemic H1N1." Influenza Other Respi. Viruses 5 Suppl. 1 (2011): 138-141. PubMed: 21761586). The failure of NR-19867 to detect recombinant H1 HA in the indirect ELISA reported here is not inconsistent with the successful use of this antibody in multi-antibody ELISA formats.

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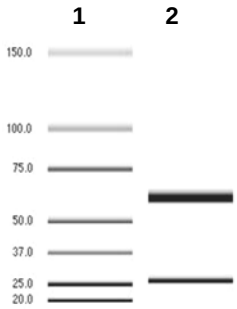
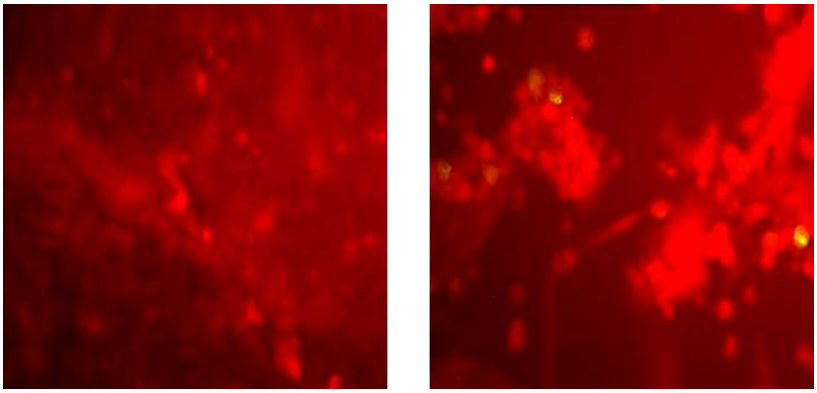
Figure 1



Lane 1: MW Markers (kDa)
Lane 2: NR-19867

Figure 2



A. Mock-infected

B. A/California/04/2009 (H1N1)pdm09-infected

Date: 29 MAY 2013

Signature: Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

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