b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-42015

Monoclonal Anti-Influenza Virus H1 Hemagglutinin (HA), A/California/04/2009 (H1N1)pdm09, Clone 1C5 (produced *in vitro*)

Catalog No. NR-42015

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

Product Description: Mouse monoclonal antibody prepared against the H1 hemagglutinin (HA) protein of the A/California/04/2009 (H1N1)pdm09 strain of influenza virus was affinity purified from clone 1C5 hybridoma supernatant using protein G magnetic beads.

Lot: 61866343 **Manufacturing Date: 25JUL2013**

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) 94.3% pure
Concentration by Spectrophotometer at OD_{280}	Report results	1.4 mg per mL
Functional Activity Indirect Immunofluorescence Assay with A/California/04/2009 (H1N1)pdm09-infected MDCK cells[1] (Figure 2) Neutralization of A/California/04/2009 (H1N1)pdm09 infectivity in MDCK cells[3]	Report results No neutralization observed	Fluorescence observed[2] No neutralization observed
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 0.01 and stained 2 days later with NR-42015 at dilutions of 1:50 and 1:500, followed by FITC-conjugated goat anti-mouse IgG F(ab')2 fragment (Millipore 5008).

[2]Clone 1C5 antibody did not function in immunofluorescence studies reported by F. Schmeisser, et al. ("Neutralizing and Protective Epitopes of the 2009 Pandemic Influenza H1N1 Hemagglutinin." Influenza Other Respir. Viruses. 7 (2013): 480-490. PubMed: 23122228.). Users should note that these authors stained influenza-infected cells that had been fixed with paraformaldehyde and permeabilized with Triton-X-100, while staining at BEI Resources was performed on acetone-fixed influenza-infected cells. Also, the concentration of NR-42015 used to obtain the result shown in Figure 2 was approximately 14-fold greater than the primary antibody concentration used by Schmeisser et al. Only very weak fluorescence was seen when a concentration of NR-42015 approximately 1.4-fold higher than that used by Schmeisser et al. was tested.

[3]100 $TCID_{50}$ of influenza virus A/California/04/2009 (H1N1)pdm09 (BEI Resources NR-13658) was pre-incubated with NR-42015 at dilutions of 1:100 and 1:500 and then used to inoculate cultures of MDCK cells (ATCC® CCL-34™). Cells were monitored for the presence of cytopathic effects for three days, and culture supernatants were tested for the presence of hemagglutinating activity. BEI Resources NR-19866 (Monoclonal Anti-Influenza Virus H1 Hemagglutinin (HA), A/California/04/2009 (H1N1)pdm09, Clone S-OIV-5H7) and NR-15511 (Monoclonal Anti-Dengue Virus Type 3 Envelope Protein, Clone E2) were used as positive and negative controls, respectively.

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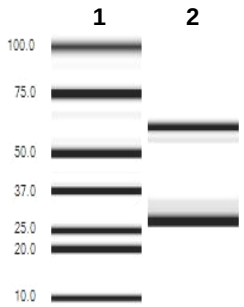
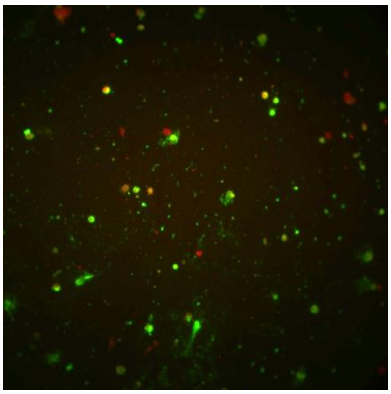
Figure 1



Lane 1: MW Markers (kDa)
Lane 2: NR-42015

Figure 2



NR-42015, 1:50 dilution

Date: 24 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.

Support Provided by NIAID

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