

Anti-Influenza A Virus Nucleoprotein antibody, mouse monoclonal (C43), pantropic to A type viruses

65-110 100 μg

Storage temperature: Ship at 4°C and store at -20°C. Avoid repeating freeze-thaw.

Specificity: Reacts with NP of all influenza A viruses so far tested, including seasonal H2N2, H3N2(A/Sydney/5/1997), and H5N1(A/crow/Kyoto53/2004), H5N1 (A/duck/Egypt/D2br10/07), H5N1(A/duck/HK/342/78), H5N2(A/crow/Kyoto/53/04), H9N1, H9N2 (A/Turkey/Wisconsin/1/66) and H1N1 (seasonal: A/New Caledonia/20/99. Pandemic: A/Suita/01/2009 and swine: A/PuertoRico/8/34). No cross reactivity with influenza B viruses.

Immunogen: Human Influenza A Virus H3N2

Applications

- 1) Western blotting (300~1,000 fold dilution) 2) Immuno-precipitation (100 fold dilution)
- 3) Immunofluorescent staining (200 fold dilution) 4) Immunohistochemistry (200 fold dilution)
- 5) ELISA (assay dependent)

Isotype: mouse IgG2A

Purity: Produced in serum-free medium and purified by proprietary chromatography procedure under mild conditions. 90~95% pure by SDS-PAGE

Form: 1 mg/ml in PBS, 50% glycerol, filter sterilized. No additive nor carrier protein added

Background: Influenza virus is an RNA virus, which causes influenza, and belongs to the family Orthomyxoviridae. Influenza virus is classified into three different genera, influenzavirus A, B, and C. They all have similar structures and compositions. The virions are 80-100nm in diameter and usually roughly spherical. The outer surface of the virion is made of a viral envelope containing two major glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Influenzavirus A is further classified into subtypes based on the surface glycoproteins, HA and NA. Currently, there are 16 HA and 9 NA subtypes. The central core of the virion contains the viral RNA genome, which is packaged in the form of ribonucleoprotein complexes.

Influenza virus nucleoprotein (NP) is a major component of the ribonucleoprotein complex and is abundantly expressed during the course of infection. It is a structural protein, which encapsidates the negative strand viral RNA and is essential for RNA transcription, replication and packaging. NP binds the PB1 and PB2 subunits of the viral RNA polymerase and the matrix protein M1, in addition to its binding to ssRNA.



NP is also known to interact with variety of other macromolecules of both viral and cellular origins, and these interactions have been shown to be essential for the viral lifecycle.

Data Link: Swiss-Prot Influenza NP

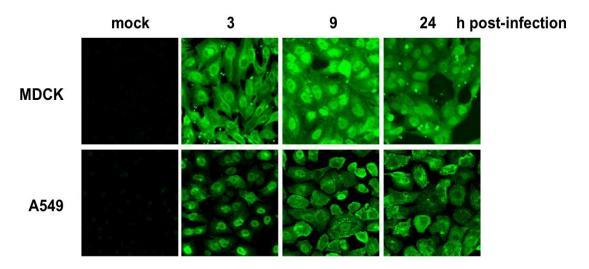


Fig.1 Immunofluorescence assay of MDCK cells derived from canine kidney cells, and A549 cells derived from human lung carcinoma cells, that were infected with H1N1 influenza virus (A/PuertoRico/8/34). Samples were taken at 3, 9, and 24 hours post-infection. C43 antibody efficiently detected virus-infected MDCK and A549 cells as early as 3 h after infection. The cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.1% 0.1% Triton X-100 in PBS. The bound antibody was visualized by a further reaction with an Alexa Fluor 488-conjugated secondary antibody

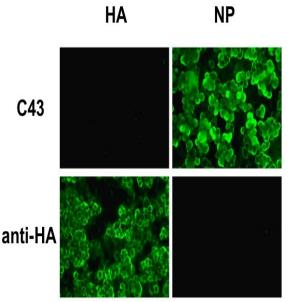


Fig.2 Immunofluorescence assay of 293T cells expressing HA or NP of pandemic (H1N1) 2009 influenza A virus (A/Suita/1/2009).

C43 specifically recognized NP-expressing cells while a commercially available mouse anti-HA monoclonal antibody specifically recognized HA.



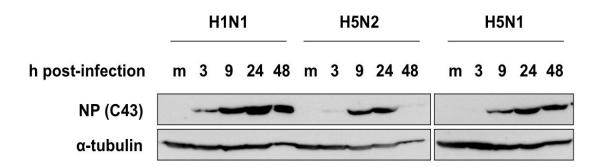


Fig.3. Western blotting of MDCK cells infected with H1N1 (A/PuertoRico/8/34), H5N1 (A/duck/HK/342/78), or H5N2 (A/crow/Kyoto/53/04) using C43 as a primary antibody. Samples were collected at 3, 9, 24, and 48 hours post-infection. C43 detected NP after 3 hours post-infection and detected three different types of influenza viruses.

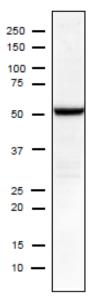


Fig.4. Identification of Influenza Nucleoprotein in crude extract of MDCK cells infected with Influenza A virus (H1N1) PuertoRico/8/34 using C43 mnoclonal antibody.

10-20% gradient gel

Blotting 15v, 30min (semi-dry)

blocking over night, 4°C

1st antibody 1/1000 dilution

2nd antibody 1/10,000 dilution; rabbit polyclonal secodary antibody to mouse IgG- H & L (HRP) (ab97046; abcam). Positions of molecular size markers are shown in kDa on the left. NP size is 56 kDa according to Swiss-Prot.

References: This antibody has been used in the following publications.

- Mizuike R. et al. Development of Two Types of Rapid Diagnostic Test Kits To Detect
 the Hemagglutinin or Nucleoprotein of the Swine-Origin Pandemic Influenza A Virus
 H1N1. Clin Vaccine Immunol 18: 494–499 (2011) PubMed ID: <u>21228147</u> (IF)
- 2. Ueda M. et al. Maturation efficiency of viral glycoproteins in the ER impacts



the production of influenza A virus. Virus Research 136: 91–97 (2008) PMID: 18550190 (WB)

- 3. Pitaksajjakul P et al. Fab MAbs specific to HA of influenza virus with H5N1 neutralizing activity selected from immunized chicken phage library. <u>Biochem Biophys Res Commun.</u> 2010 May 14;395(4):496-501. PMID: <u>20382115.</u> (IF)
- 4. Okuno Y *et al*. A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains. J Virol 67: 2552–2558 (1993) _PubMed ID:7682624 (IP)