ROCKLAND
Immunochemicals for Research

800-656-7625

fax 610-367-7825

Certificate of Analysis

Product: Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-2) (Protein A Purified)

Code: 200-301-975 **Lot #:** 19009

Size: 100 µg **Physical State:** Liquid (sterile filtered)

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None **Preservative:** 0.01% (w/v) Sodium Azide

Clone: 15A3 ($IgG_{2a\kappa}$) **Fusion Partner:** Sp2/0 - Ag14

Storage Conditions: Store vial at -20° C or below prior to opening. Dilute only prior to immediate use. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening product.

Background Information: Antibody raised against the hemagglutinin (HA) surface glycoprotein of the A/Vietnam/1203/04 (H5N1) influenza virus. Generally referred to as "bird flu", the H5N1 influenza A virus has been documented in poultry and humans across ten Eurasian countries, from Japan in the north to Indonesia in the south. Without immunity, humans would have no protection against H5N1 influenza viruses, which could potentially cause a catastrophic pandemic influenza. This antibody, directed against the HA surface glycoprotein of the A/Vietnam/1203/04 (H5N1) influenza virus, is intended to further our understanding of the mechanisms underlying antigenic variation and evolution of novel variants. The major functions of HA include receptor-binding and fusion activities, but there may also be a structural role for HA in viral particle formation. Following attachment of HA to surface receptors on susceptible cells, the influenza virus enters the cell via endocytosis and membrane fusion.

Application Note(s): This monoclonal antibody can be used for hemagglutination inhibition (HI) assays to provide antigenic characterization of the influenza A viruses of the H5 HA subtype. This monoclonal antibody is suitable for virus neutralization assays (in cell culture and in embryonated chicken eggs), ELISA, immunoprecipitation, immunohisto-chemistry and western blotting.

Recommended Dilutions:

HEMAGGLUTINATION INHIBITION ASSAY	1:100 - 1:4,000
NEUTRALIZATION ASSAY	1:10,000 - 1:50,000
ELISA	1:5,000
WESTERN BLOT	User Optimized
IMMUNOPRECIPITATION	User Optimized
IMMUNOHISTOCHEMISTRY	User Optimized
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This product was purified from tissue culture supernatant fluid by Protein A chromatography and is specific for H5 hemagglutinin (HA) protein of influenza A virus [strain A/Vietnam/1203/04 (H5N1)]. VN04-2 monoclonal antibody did not cross-react with influenza viruses of other HA subtypes. This monoclonal antibody reacted with H5N1 influenza viruses representatives of different clades and subclades of the H5 HA subtype.

Immunogen: This monoclonal antibody was produced by intraperitoneal immunization of BALB/c mice with concentrated purified virus preparation containing hemagglutinin (HA) protein of influenza A virus [strain A/Vietnam/1203/04 (H5N1)] using the modification of the method described by Kohler and Milstein. Each mouse received two immunizations of 15 µg HA with incomplete Freund's adjuvant, administered 3 week apart.

Relevant Links: NCBI AY818135 Virion structure

Related Product(s):

#500-301-975 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-2) Ascites
#500-301-976 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-8) Ascites
#500-301-977 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-9) Ascites
#500-301-978 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-10) Ascites
#500-301-979 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-13) Ascites
#500-301-980 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-16) Ascites
#200-301-975 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-2) (Protein A Purified)
#200-301-976 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-8) (Protein A Purified)
#200-301-977 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-9) (Protein A Purified)
#200-301-978 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-10) (Protein A Purified)
#200-301-979 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-13) (Protein A Purified)
#200-301-980 Mouse Monoclonal Anti-H5 Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-16) (Protein A Purified)
#610-703-124 HRP Anti-Mouse IgG [H&L] MX10 (DONKEY)
#610-132-121 IRDye800 Anti-Mouse IgG [H&L] MX10 (GOAT)

Specific References:

Kohler G and Milstein C. (1976) Derivation of Specific Antibody-Producing Tissue Culture and Tumor Lines by Cell Fusion. *Europ*. *J. Immunol.* **6:** 511-519.

Govorkova, E.A., et al. (2006) Immunization with Reverse-Genetics–Produced H5N1 Influenza Vaccine Protects Ferrets against Homologous and Heterologous Challenge. *J. Infect. Dis.* **194**:159–167.

Hoffmann, E., et al. (2005) Role of Specific Hemagglutinin Amino Acids in the Immunogenicity and Protection of H5N1 Influenza Virus Vaccines. *Proc. Natl. Acad. Sci.* **102**(36): 12915–12920.

General References:

Guan, Y., et al. (2004) H5N1 Influenza: A Protean Pandemic Threat. *Proc. Natl. Acad. Sci. U.S.A.* **101**: 8156–8161.

Li, K. S., et al. (2004) Genesis of a Highly Pathogenic and Potentially Pandemic H5N1 Influenza Virus in Eastern Asia. *Nature* **430**: 209–213.

Stevens, J., et al. (2006) Structure and Receptor Specificity of the Hemagglutinin from an H5N1 Influenza Virus. *Science* **312**: 404–410.

Hatta, M., et al. (2001) Molecular Basis for High Virulence of Hong Kong H5N1 Influenza A Viruses. *Science* **293**: 1840–1842.

Webster, R.G., et al. (1980) The Mechanism of Antigenic Drift in Influenza Viruses: Analysis of Hong Kong (H3N2) Variants with Monoclonal Antibodies to the Hemagglutinin Molecule. *Ann NY Acad Sci*. **354**:142-161.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.