

Datasheet for 200-301-975**H5N1 Antibody VN04-2****Overview**

Description:	H5N1 Antibody (VN04-2) - 200-301-975
Item No.:	200-301-975
Size:	100 µg
Applications:	Neutralization, ELISA, FC, WB
Reactivity:	Virus
Host Species:	Mouse

Product Details

Background:	Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-2) 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.
Synonyms:	mouse anti-H5N1 Antibody, mouse anti-VN04-2 Antibody, H5HA antibody, Hemagglutinin 5 antibody, H5N1 antibody
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	15A3
Format:	IgG2a

Target Details

Gene Name:	HA
-------------------	----

Reactivity:	Virus
Immunogen Type:	Native Protein
Immunogen:	A/Vietnam/1203/04 Influenza Virus (VN04-2) 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.
Purity/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.
Relevant Links:	• NCBI - 58618437 • NCBI - 159144921 • UniProtKB - A8UDQ2

Application Details

Tested Applications:	Neutralization
Suggested Applications:	ELISA, FC, WB (Based on references)
Application Note:	Hemagglutinin of A/Vietnam/1203/04 Influenza Virus (VN04-2) 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, immunohistochemistry and western blotting.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000
IHC:	User Optimized
IP:	User Optimized
Neutralization:	User Optimized
WB:	User Optimized

Formulation

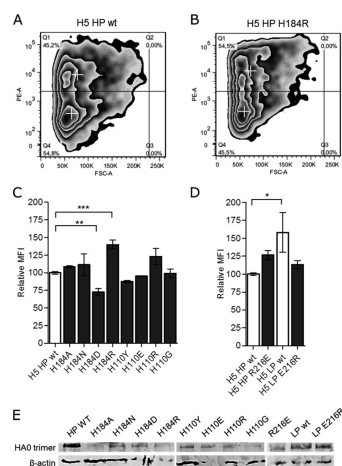
Physical State:	Liquid (sterile filtered)
------------------------	---------------------------

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

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



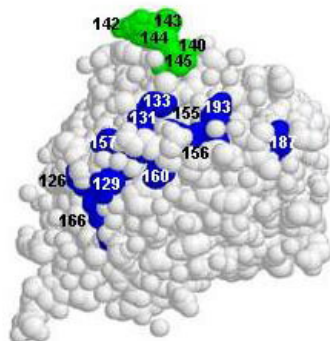
Flow Cytometry

Cell surface expression of wild-type and mutant HA proteins of H5 HP and LP. Cell surface expression of mutant proteins was quantified by immunostaining and flow cytometry using the H5-specific antibody Vn04-2 (p/n 200-301-975).

(A and B) Representative images of flow cytometry measurements using the highly pathogenic wild-type protein (A) and the H184R mutant protein (B). Gate Q1 represents the fluorescent positive cells of each measurement compared to the mock control, with red crosses indicating the median fluorescence intensity of the respective gate. FSC-A, forward scatter, area; PE-A, phycoerythrin, area. (C and D) The median fluorescence intensity (MFI) value of fluorescent cells (gate Q1) in each sample was normalized to that of the H5 HP HA wild-type protein (relative MFI). Error bars represent the standard errors of the means from triplicate experiments. Significant differences are marked by asterisks with probability determined by one-way ANOVA and Dunnett's multiple comparison posttest: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (E) Trimer formation of histidine mutants and of highly pathogenic and low pathogenic H5 HA with mutation at position 216. HA trimers and the cellular protein β -actin were detected by SDS-PAGE and Western blotting following incubation with the cross-linking agent DSP. Fig2. PMID: 25187542

Diagram

Schematic representation of the antigenic sites and the epitopes on the globular head of the HA H5 HA molecule. Images were created with RasMol 2.6, and the HA structure was obtained from the Protein Data Bank (PDB accession number 1JSM). Amino acid positions are designated in H3 numbering. Image provided courtesy of Elena Govorkova Ph D.



References

- Kaverin NV. et al. Pleiotropic effects of amino acid substitutions in H5 hemagglutinin of influenza A escape mutants. *Virus Res.* (2015)
- Mair CM. et al. A histidine residue of the influenza virus hemagglutinin controls the pH dependence of the conformational change mediating membrane fusion. *J Virol.* (2014)
- Reed ML. et al. Amino acid residues in the fusion peptide pocket regulate the pH of activation of the H5N1 influenza virus hemagglutinin protein. *J Virol.* (2009)
- Lim APC. et al. Neutralizing human monoclonal antibody against H5N1 influenza HA selected from a Fab-phage display library. *Viol J.* (2008)
- Kaverin NV. et al. Epitope Mapping of the Hemagglutinin Molecule of a Highly Pathogenic H5N1 Influenza Virus by Using Monoclonal Antibodies. *ASM Journals Journal of Virology* (2007)
- Hanson BJ. et al. Passive immunoprophylaxis and therapy with humanized monoclonal antibody specific for influenza A H5 hemagglutinin in mice. *Respiratory Research* (2006)

Disclaimer

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. 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. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.