

Certificate of Analysis

Product: Mouse Monoclonal Anti-Mouse Hepatitis Virus (Strain A59) Nonstructural Protein 9 (nsp9) (Protein A Purified)

Code: 200-301-A56

Lot #: 19222

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 2.1 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: 2C6.H1 (IgG_{2bκ})

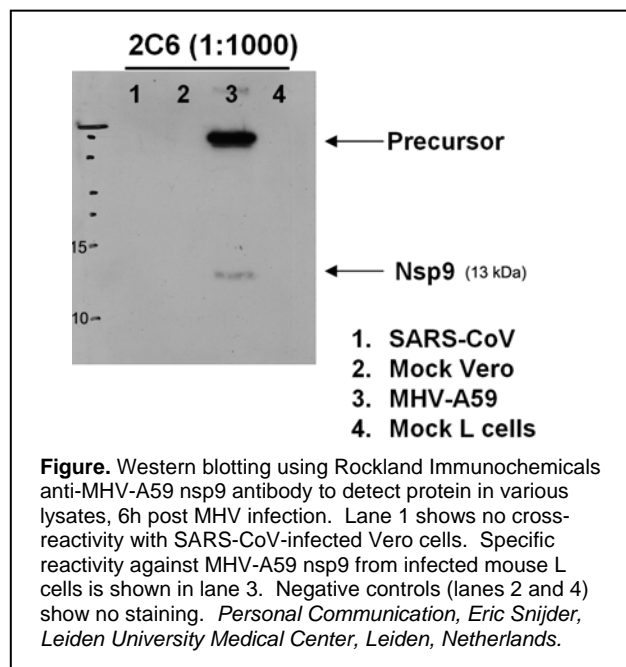
Fusion Partner: Sp2/0 - Ag14

Storage Conditions: Store vial at -20° C 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.

Background Information: The nonstructural protein 9 (nsp9) is one of the Mouse hepatitis virus replicase cleavage products, encoded by ORF1a. Nsp9 is an RNA-binding protein that is thought to be part of the viral replication complex, which is associated with intracellular membranes.

Recommended Dilutions:

WESTERN BLOT	1:1,000
IF MICROSCOPY	1:1,000
IMMUNO PRECIPITATION	User Optimized
OTHER APPLICATIONS	User Optimized



Application Note(s): This antibody has been tested for use in immunofluorescence microscopy and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 13 kDa in size corresponding to mature MHV-A59 nsp9 by western blotting in the appropriate cell lysate or extract. For immunofluorescence microscopy, Vero-E6 cells were grown on glass slides followed by infection with MHV-A59 strain and fixation with PBS/3%PFA. After washing and permeabilization of the fixed cells, antibody incubation was performed in PBS/5%FCS for 30 min.

Relevant Link(s): NCBI Link (polyprotein) [NP_068668](https://pubmed.ncbi.nlm.nih.gov/16482221/)

NCBI [MHV-A59](https://pubmed.ncbi.nlm.nih.gov/16482221/)

Purity and Specificity: This antibody is directed against the MHV-A59 nsp9 protein. This product was purified from tissue culture supernatant fluid by Protein A chromatography. No cross reactivity occurs with SARS CoV nsp9. Cross reactivity with homologues from other sources has not been tested.

Immunogen: This antibody was produced in mice by repeated immunizations with *E.coli* derived full-length MHV-A59 nsp9 protein. This protein is part of the viral replicase polyprotein.

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.

General References:

Ziebuhr, J., Snijder, E. J., and Gorbalenya, A. E. (2000). Virus-encoded proteinases and proteolytic processing in the Nidovirales. *J. Gen. Virol.* **81**:853-879.

Snijder, E. J., Bredenbeek, P.J., Dobbe, J.C., Thiel, V., Ziebuhr, J., Poon, L. L. M., Guan, Y., Rozanov, M., Spaan, W. J. M. and Gorbalenya, A. E. (2003) Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J. Mol. Biol.* **331**:991-1004.

Egloff, M. P., Ferron, F., Campanacci, V., Longhi, S., Rancurel, C., Dutartre, H., Snijder, E. J., Gorbalenya, A. E., Cambillau, C. and Canard, B. (2004). The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. *Proc. Natl. Acad. Sci. U. S. A.* **101**:3792-3796.

Sutton, G., Fry, E., Carter, L., Sainsbury, S., Walter, T., Nettleship, J., Berrow, N., Owens, R., Gilbert, R., Davidson, A., Siddell, R., Poon, L.L. Diprose, J., Alderton, D., Walsh, M., Grimes, J.M. and Stuart, D.I. (2004) The nsp9 replicase protein of SARS-coronavirus, structure and functional insights. *Structure (Camb.)* **12**:341-353.

Bost, A. G., Carnahan, R. H., Lu, X. T. and Denison, M. R. (2000) Four proteins processed from the replicase gene polyprotein of mouse hepatitis virus colocalize in the cell periphery and adjacent to sites of virion assembly. *J. Virol.* **74**:3379-3387.

Related Products:

# 200-401-A50 Protein A Purified Anti-SARS-CoV (N) Protein (Rabbit)	#200-401-A54 Protein A Purified Anti-SARS-CoV nsp13 (Rabbit)
# 200-401-A51 Protein A Purified Anti-SARS-CoV 3CL Protease (Rabbit)	#200-401-A55 Protein A Purified Anti-SARS-CoV (M) Protein (Rabbit)
#200-401-A52 Protein A Purified Anti-SARS-CoV nsp3 (Rabbit)	# 611-103-122 HRP Anti-RABBIT IgG (H&L) (GOAT) MX10
#200-401-A53 Protein A Purified Anti-SARS-CoV nsp8 (Rabbit)	# 611-132-122 IRDye800 Anti-RABBIT IgG (H&L) (GOAT) MX10
# MB-070 Blocking Buffer for Fluorescent Western Blotting	# KIA-003 MaxTag [™] Anti-RABBIT IgG Kit for Immunoblotting

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.

Figure 2. Immunofluorescence microscopy using Rockland Immunochemical's anti-MHV-A59 nsp9 antibody, 6-h post infection in mouse L cells. Cells were fixed in 3% para-formaldehyde. For detection Cy2 conjugated Goat-anti-Mouse IgG MX10 (610-111-121) was used. *Personal Communication, Eric Snijder, Leiden University Medical Center, Leiden, Netherlands.*

