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User's Manual

Mouse Anti-Norovirus (MNV1/VP1) IgG ELISA Kit

REF **DEIANS084**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Mouse Anti-Norovirus IgG (MNV1) ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for Norovirus strain 1 major capsid protein (VP1) antigen in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. The kit has no inactivated virus or viral proteins to avoid infection. The antigen used is highly purified recombinant VP1 protein. This kit is for research use only (RUO).

General Description

Mouse norovirus (MNV), a non-enveloped ss-RNA virus (Calciviridae) and the most prevalent viral infection in laboratory animal facilities, is highly contagious in causing a mild, persistent enteric infection. MNV replicates in macrophages and dendritic cells, with the potential to alter research data. The major capsid protein, VP1, is highly immunogenic and is implicated in viral virulence in mammals. MNV infection may be diagnosed by ELISA, measuring rapidly rising antibody titers (8-12 days after infection) to MNV antigen. Mice infected with MNV are not suitable for animal research; in addition to lung changes, MNV may predispose to secondary bacterial infection, cause infertility, and death in susceptible strains. Besides infecting animals, MNV may also contaminate cell lines, transplantable tumors and other biological products; these should be tested by mouse antibody production (MAP), using ELISA to detect anti-MNV after immunization.

Principles of Testing

The Mouse Anti-MNV VP1 IgG ELISA kit is based on the binding of mouse anti-MNV IgG in samples to MNV antigen immobilized on the microwells, and anti- MNV IgG antibody is detected by anti-mouse IgG-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of antibody present in the sample. Stopping Solution is added to terminate the reaction (convert blue to yellow color). Absorbance at 450nm is then measured using an ELISA reader. The presence of mouse IgG antibody in samples is determined relative to mouse anti-MNV IgG Controls.

Reagents And Materials Provided

1. MNV Ag Microwell Strip Plate, 8-well strips (12), Coated with MNV VP1 antigen, and postcoated with stabilizers. **2. Mouse Anti-MNV Sensitivity Control,** 0.65 ml, Low level Mouse AntiMNV, diluted in buffer with protein, detergents and antimicrobial. **3. Mouse Anti-MNV Positive Control,** 0.65 ml, High level Mouse AntiMNV, diluted in buffer with protein, detergents and antimicrobial. **4. Low NSB Sample Diluent,** 30 ml, Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. **5. TMB Substrate,** 12 ml, Chromogenic substrate for HRP containing TMB and peroxide. **6. Stop Solution,** 12 ml, Dilute sulfuric acid. **7. Wash Solution Concentrate (100×),** 10 ml **8. Sample Diluent Concentrate (20×),** 10 ml **9. Anti-Mouse IgG HRP Conjugate Concentrate (100×),** 0.15 ml

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended. 2. Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Mouse IgG HRP Concentrate. Stock bottle to store diluted Wash Solution; 0.2 to 1L. 3. Distilled or deionized water to dilute reagent concentrates. 4. Microwell plate reader at 450 nm wavelength.

Storage

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

Specimen Collection And Preparation

Sample Collection and Handling Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage. **Antibody Stability** Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay. Example: Initial (1/5): 10µl serum + 40µl WSD [or 0.1ml + 0.4ml] Further (1/100): 10µl initial (1/5) + 190µl LNSD **Assay Design** Review Calculation and Limitations before proceeding: 1. Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the Sensitivity Control. This is usually 1/50 or greater dilution for mouse sera with normal levels of IgG and IgM. 2. Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3. 3. Run the Positive and Sensitivity Controls, which validate that the assay was performed to specifications: the Positive Control should give a high signal (>1.5 OD); the Sensitivity Control should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results).

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes). 1. Determine the number of wells for the assay run. Duplicates are recommended, including 4 Control wells and 2 wells for each sample and internal control to be assayed. 2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated. 3. Add 200-300µl Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Reagent Preparation

1. Wash Solution Concentrate (100×): Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at ambient temperature until kit is

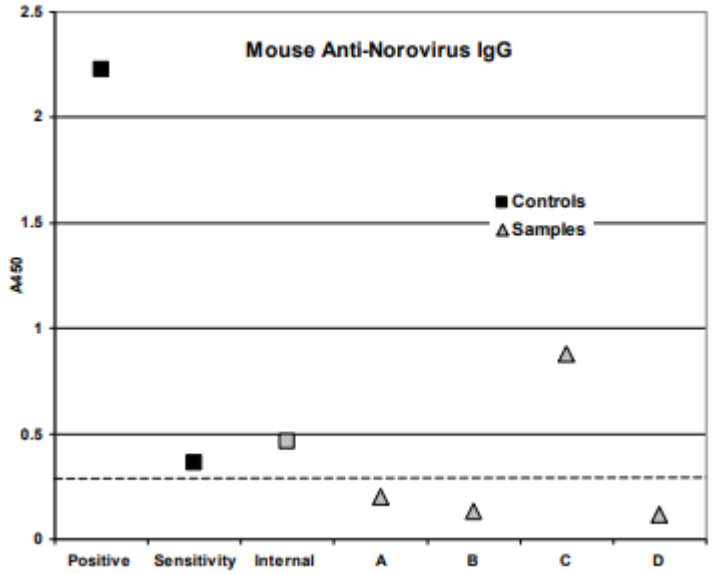
used entirely. **2. Sample Diluent Concentrate (20×):** Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up. **3. Anti-Mouse IgG HRP Conjugate Concentrate (100×):** Peroxidase conjugated anti-Mouse IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10µl of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100× to 2-8°C storage.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation. 1. 1st Incubation [100µl - 60 min; 4 washes] a. Add 100µl of calibrators, samples and controls each to predetermined wells. b. Tap the plate gently to mix reagents and incubate for 60 minutes. c. Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility. **2. 2nd Incubation [100µl-30 min; 5 washes]** a. Add 100µl of diluted Anti-Mouse IgG HRP to each well. b. Incubate for 30 minutes. c. Wash wells 5 times as in step 2. **3. Substrate Incubation [100ul-15 min]** a. Add 100µl TMB Substrate to each well. The liquid in the wells will begin to turn blue. b. Incubate for 15 minutes in the dark, e.g., place in a drawer or closet. **Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid). 4. Stop Step [Stop: 100µl]** a. Add 100µl of Stop Solution to each well. b. Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow. **5. Absorbance Reading** a. Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available. b. Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

Calculation

A. Antibody Activity Threshold Index Compare Samples to Sensitivity Control or Internal Control = Positive/Negative Cut-off. Example:



Results: The sensitivity of the assay to detect anti-MNV IgG, from either natural infection or MAP, is controlled so that the

Sensitivity Control represents a threshold OD for most true positives in mouse serum diluted in the Low NSB Sample Diluent at 1:100 or greater. Visual inspection of the data in the above graph shows the following: **Positive Control** – clearly positive, shows the OD range of the assay; high value indicates optimal sensitivity of the assay. **Sensitivity Control** – a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. The is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives. **Internal Control** – a true positive from an infected animal that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the Sensitivity Control for Positive/Negative discrimination purposes. **Samples A,B,C,D** – 3 samples (A, B, D) are negative: below the threshold; 1 sample (C) is positive: clearly above the threshold. The Sensitivity Control can be used to calculate a Threshold Index that numerically discriminates Positive/Negative, as follows: Divide each Sample net OD by the Sensitivity Control net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody. **B. Positive Index** Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows: 1. Calculate the net OD mean + 2 SD of the Control/Nonimmune samples = Positive Index. 2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody. A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution. This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa. Example:

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.248	**P** 2.212	0.79	**7.04**
2	0.290	**S** 0.452	0.92	**1.44**
3	0.186	**I** 0.541	0.59	**1.72**
4	0.276	**U** 0.212	0.88	0.68
5	0.161	**U** 0.122	0.51	0.39
6	0.173	**M** 1.491	0.55	**4.75**
7	0.153	**M** 0.694	0.48	**2.21**
8	0.211	**N** 1.487	0.67	**4.74**
9	0.145	**N** 0.546	0.46	**1.74**
10	0.110	**U** 0.263	0.35	0.84
Mean	0.195			
SD	0.0595			
Mean +2 SD	**0.314**	= **Positive Index**		

Results: **Experimental Samples** are represented as follows: **P** – Positive Control **S** – Sensitivity Control **I** – Internal Control; lab's threshold positive serum **U** – Uninfected mouse sample **M** – Mouse Antibody Production (MAP) samples represent injection of MNV antigen (same as used for plate coating) into mice; positive indicates presence of the MNV in the inoculum. **N** – Naturally infected mouse samples. **Note: The prevalent IgG antibody from MAP samples can be a different subclass (often IgG1, depending on adjuvant used) from the prevalent subclass from natural infection (IgG2a). The Anti-IgG-HRP used in the kit has been balanced for equal sensitivity for the IgG1 and IgG2a subclasses, therefore avoiding a bias in assay sensitivity for the various uses of the assay.**

Precision

Samples and Controls were assayed in duplicate in 5 - 6 separate runs, to provide a measure of between-assay reproducibility. The data are represented using the value of the Sensitivity Control in each assay to calculate a Threshold Index for each control and samples.

Sample	Ave OD	Threshold Index (mean)	Inter-assay %CV
Positive Control	2.22	7.78	**2.2**
Sensitivity Control	0.36	**1.0**	**0**
Internal Control	0.46	1.35	**2.9**
MAP Positive	0.88	2.89	**5.0**

Results: The coefficient of variation (%CV) shows the reproducibility of the assay for measuring one antibody activity (sample or control) relative to another antibody activity (Sensitivity Control). Variation increases in the threshold region; for this reason, consider running additional tests for borderline samples.

Sensitivity

The MNV antigen coating level, HRP conjugate concentration, and Low NSB Sample Diluent are optimized to differentiate anti-MNV IgG from background (non-antibody) signal with mouse serum samples diluted 1:100. The Anti-Mouse IgG conjugate is blended to equally quantify IgG1 and IgG2a subclass antibody, important when considering difference in subclass emphasis between natural infection and MAP immunization.

Specificity

Purified recombinant protein (E.coli) of the Norovirus-1 major capsid protein (VP1) is used to coat the microwells; thus the assay is specific for antibodies directed to MNV-1 VP1 protein. The Anti-Mouse IgG HRP conjugate reacts specifically with mouse IgG class antibodies. IgA, IgM and IgE antibody would not be measured above background signals.

Precautions

Controls, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes.

Limitations

1. The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a Positive Index, or by using known Internal Controls as discriminator for a Threshold Control (instead of the kit Sensitivity Control) 2. The assay

detects and quantifies IgG antibodies directed to the major capsid protein VP1. It may be possible for an animal to have norovirus infection without producing antibodies specific to VP1. 3. Anti-MNV antibody levels of an infected animal may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.