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User's Manual

Monkey Anti-Norovirus GI.1 VP1 IgG ELISA Kit

REF **DEIANS087**

Σ **96T**

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

The Monkey anti- Norovirus GI.1 VP1 IgG ELISA Kit is an indirect ELISA suitable for detecting and quantifying IgG antibody activity specific for Norovirus GI.1 VP1 in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. The kit is particularly suited to assess the efficacy of various Norovirus vaccines in monkey model. For research use only (RUO).

General Description

Noroviruses are a genetically diverse group of viruses belonging to the family Caliciviridae. and are the most common cause of gastroenteritis in humans. Noroviruses can genetically be classified into at least seven different genogroups (GI, GII, GIII, GIV, GV, GVI, and GVII). Genogroups I, II and IV infect humans, genogroup III infects bovine species, and genogroup V has recently been isolated in mice. Most noroviruses that infect humans belong to genogroups GI and GII, with genogroup II, genotype 4 (GII.4) accounting for the majority of adult outbreaks of gastroenteritis. Noroviruses contain a linear, non-segmented, positive-sense RNA genome of approximately 7.5 kilobases encoding a large polyprotein which is cleaved into six smaller non-structural proteins (NS1/2 to NS7) by the viral 3C-like protease (NS6), a major structural protein, VP1, of about 58~60 kDa and a minor capsid protein (VP2).

Principles of Testing

The Monkey Anti-NGI.1 IgG ELISA kit is based on the binding of antibody in samples to NGI.1 (serotype specific) antigen immobilized on the microwells. Bound antibody is then detected by antibody-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate which is directly proportional to the amount of antibody present in the sample. Stopping Solution is added to terminate the reaction and absorbance at 450nm is then measured using an ELISA microwell reader. The presence of Monkey NGI.1 IgG antibody in samples is determined relative to anti-NGI.1 Calibrators supplied in the kit

Reagents And Materials Provided

1. Norovirus GI.1 VP1 Coated Strip Plate, 8-well strips (12), Coated with recombinant NGI.1 VP1 protein, and postcoated. 2. Anti-Norovirus GI.1 VP1 Positive Control, 0.65 ml, Serum with anti-Norovirus GI.1 VP1 reactivity; [Value Range on Label] 3. Anti-Norovirus GI.1 VP1 Calibrator 1 U/ml, 0.65 ml 4. Anti-Norovirus GI.1 VP1 Calibrator 2.5 U/ml, 0.65 ml 5. Anti-Norovirus GI.1 VP1 Calibrator 5 U/ml, 0.65 ml 6. Anti-Norovirus GI.1 VP1 Calibrator 10 U/ml, 0.65 ml

Four (4) vials, each containing anti-Norovirus GI.1; in buffer with antimicrobial.

7. Anti-Monkey IgG HRP Conjugate (100×), 0.15 ml 8. Sample Diluent (20×), 10 ml 9. Low NSB Sample Diluent, 30 ml, Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. 10. Wash Solution Concentrate (100×), 10 ml 11. TMB Substrate, 12 ml, Chromogenic substrate for HRP containing TMB and peroxide. 12. Stop Solution, 12 ml, Dilute sulfuric acid. 13. Product Manual, 1 ea

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml. 2. Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Monkey IgG HRP Concentrate. 3. Stock bottle to store diluted Wash Solution; 0.2 to 1L. 4. Distilled or deionized water to dilute reagent concentrates. 5. Microwell plate reader at 450 nm wavelength and ELISA plate washer (if available)

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 & Dilution** 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 Further (1/100): 10µl initial (1/5) + 190µl LNSD (1/100) **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 1 U/ml Calibrator. This is usually 1:100 or greater dilution for monkey serum/plasma with normal levels of IgG and IgM. 2. Run the Anti- Norovirus GI.1 VP1 Positive Control; value range is on the label. 3. 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. 4. Run a set of Calibrators, which validate that the assay was performed to specifications: 10 U/ml should give a high signal (>1.5 OD); 1 U/ml should give a low signal which can be used to discriminate at the Positive/Negative threshold.

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 8 Calibrator wells and 2 wells for each sample 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 1-5 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 refrigerated for long term and

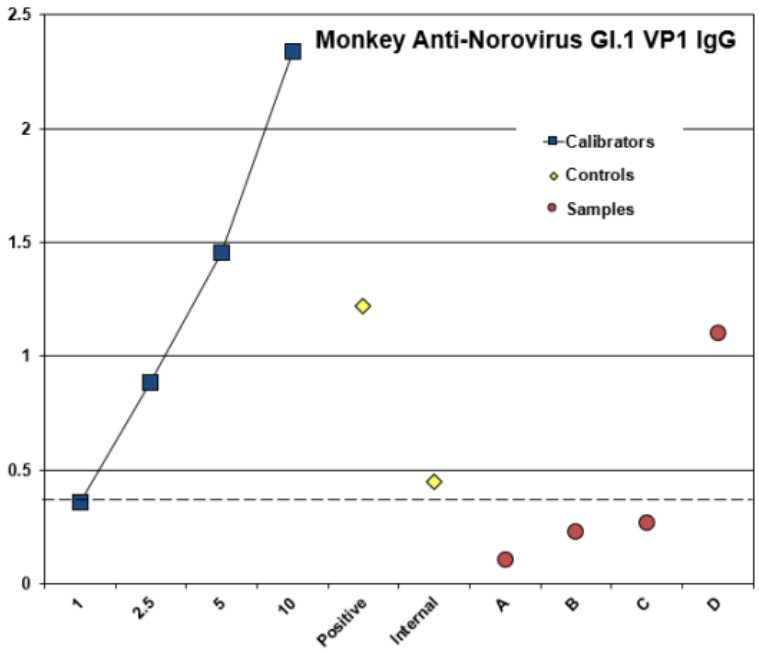
ambient temp. for short term. **2. Sample Diluent Concentrate (20×):** Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up. **3. Anti-Monkey IgG HRP Conjugate Concentrate (100×):** Peroxidase conjugated anti-monkey 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 pre-determined 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-Monkey IgG HRP to each well. b. Incubate for 30 minutes. c. Wash wells 5 times as in step 1. **3. Substrate Incubation [100µl-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

Method A. Antibody Activity Threshold Index Compare Samples to 1 U/ml Calibrator or Internal Control **= Positive/Negative Cut-off.** Example:



Results: The sensitivity of the assay to detect anti-NGI.1 IgG, from either natural exposure or vaccination, is controlled so that the 1 U/ml Calibrator represents a threshold OD for most true positives in monkey serum diluted to 1:100 or less. Visual inspection of the data in the above graph shows the following: **Calibrators** – dilution curve of an anti-NGI.1 antibody, derived from NGI.1 vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay. **1 U/ml:** a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives. **Positive Control** – an anti-NGI.1 serum; value range is on the label. The control can be used to gauge reproducibility and to normalize between-assay variation. **Internal Control** – a low level positive from an immunized individual that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes. **Samples A,B,C,D,** – 3 samples (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold. The 1 U/ml Calibrator can be used to calculate a Threshold Index that numerically discriminates Positive/Negative: Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody. **Method 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 non-immune 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:

Sample	Assay Net OD		Calculated Antibody Activity	
	Control	Exptl	Control	Exptl
1	0.244	**C** 2.293	0.57	**5.34**
2	0.204	**C** 1.490	0.48	**3.47**
3	0.237	**C** 0.833	0.55	**1.94**
4	0.26	**C** 0.326	0.61	0.76
5	0.388	**P** 1.106	0.90	**2.58**
6	0.407	**I** 0.310	0.95	0.72
7	0.288	**E** 0.672	0.67	**1.56**
8	0.263	**E** 0.363	0.61	0.85
9	0.322	**E** 0.560	0.75	**1.31**
10	0.343	**E** 0.490	0.80	1.14
Mean	0.295			
SD	0.067			
Mean +2 SD	**0.429**	= **Positive Index**		

Results: Experimental Samples are represented as follows: **C** – Calibrator **P** – Positive Control **I** – Internal Control; lab's threshold positive serum **E** – Experimental sample **Method C. Titers from Sample Dilution Curves** The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines: 1. Use an OD value Index in the mid-range of the assay (2.0-0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used. 2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample. 3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data. 4. The Positive and Sensitivity Control values can be used to normalize inter-assay values. Calculations: 1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator). 2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index = IgG Antibody Activity Units

Specificity

Norovirus GI.1 protein VP1 virus-like particles, expressed in insect cells and purified from baculovirus infected cell pellets, are used to coat the microwells; thus, no other antibody specificity is detectable in the assay. The Anti-Monkey IgG HRP conjugate reacts specifically with monkey IgG class antibodies; IgM, IgA, and IgE antibodies would not be measured above background signals.

Precautions

Calibrators, 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. All reagents may be disposed of down a drain with copious amounts of water.

Limitations

1. The assay detects and quantifies IgG antibodies directed to the VP1 protein. Individuals may be exposed to the virus without producing antibodies specific to VP1. 2. Anti-NGI.1 antibody levels of an immunized host may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development. 3. 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 1 U/ml Calibrator Control)