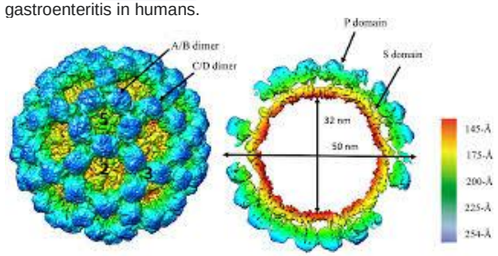
INTENDED USE

The **Human anti- Norovirus GII.4 VP1 IgM** ELISA Kit is an indirect ELISA suitable for detecting and quantifying IgM antibody activity specific for Norovirus GII.4 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 human model. For research use only (RUO).

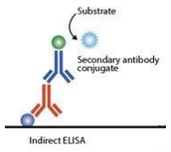
GENERAL INFORMATION

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**).

PRINCIPLE OF THE TEST



The Human Anti-NGII.4 IgM ELISA kit is based on the binding of antibody in samples to NGII.4 (serotype specific) antigen immobilized on the microwells, and bound antibody is 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 Human NGII.4-IgM antibody in samples is determined relative to anti-NGII.4 Calibrators supplied in the kit.

PRODUCT SPECIFICATIONS

Specificity
Norovirus GII.4 protein VP1 virus-like particles, expressed in and purified from HEK293 cells, are used to coat the microwells; thus, no other antibody specificity is detectable in the assay. The Anti-Human IgM HRP conjugate reacts specifically with human IgM class antibodies; IgG, IgA and IgE antibodies would not be measured above background signals.

KIT CONTENTS

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.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	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.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	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.
Anti-Human IgM-HRP Conjugate Concentrate (100x) Part: H-HuM.2a11, 0.15ml	Peroxidase conjugated anti-human IgM in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Norovirus GII.4 VP1 Coated Strip Plate	NG2001	8-well strips (12)	Coated with recombinant NGII.4 VP1 protein and post-coated.
Anti-Norovirus GII.4 VP1 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	NG2002B NG2002C NG2002D NG2002E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials each containing anti-Norovirus GII.4; in buffer with antimicrobial.
Anti-Norovirus GII.4 VP1 Positive Control	NG2002-PC	0.65 ml	Serum with anti-Norovirus GII.4 VP1 reactivity. [Value Range on Label]
Low NSB Sample Diluent	TBTm **Not** for HRP dilution	30 ml	Buffer with protein, detergents, and anti-microbial. Use as is for sample dilution
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:
- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgM HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength and ELISA plate washer (if available)

ASSAY DESIGN AND SET-UP

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): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/100): **10**ul initial (1/5) + **190**ul LNSD (1/100)

Assay Design
Review Interpretation of Results and Limits of the Assay (p.5-7) before proceeding:

- 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 human serum/plasma with normal levels of IgG and IgM.
- Run the Anti- **Norovirus GII.4 VP1 Positive Control**; value range is on the label.
- 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.
- 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 (see Interpretation of Results, p. 5).

Plate Set-up
Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- 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.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul 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.

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 [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- 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 [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgM HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- 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: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- 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.
- 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.

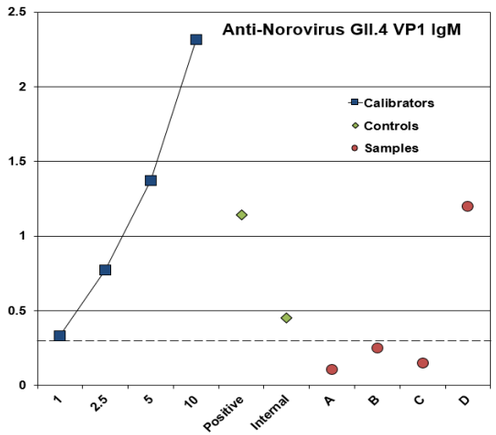
INTERPRETATION OF RESULTS

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-NGII.4 IgM, from either natural exposure or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or more. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti- NGII.4 antibody, derived from NGII.4 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-NGII.4 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.

INTERPRETATION OF RESULTS (cont)

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/Non-immune 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:

	Assay Net OD		Calculated Antibody Activity	
Sample	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

ASSAY PERFORMANCE

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

   = **IgM Antibody Activity Units**

Limits of the Assay

- The assay detects and quantifies IgM antibodies directed to the VP1 protein. Individuals may be exposed to the virus without producing antibodies specific to VP1.

- Anti- NGII.4 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.

- 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** (page 6), or by using known **Internal Controls** as discriminator for a **Threshold Control** (instead of the kit 1 U/ml Calibrator Control)

PRECAUTIONS AND SAFETY INSTRUCTIONS

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. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

Instruction Manual No. M-NG2-HIM-055

Human Anti-Norovirus GII.4 VP1 IgM ELISA Kit

Cat. #NG2-HIM-055; 96 tests

For the Detection and Quantitation of Anti-Norovirus GII.4 VP1 IgM in Human Serum/Plasma

For research use only, not for diagnostic or therapeutic use.

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ELISA Kit Components	Amount	Part
Norovirus GII.4 VP1 Coated Strip Plate	8-well strips (12)	NG2001
Anti-Norovirus GII.4 VP1 Positive Control	0.65 ml	NG2002PC
Anti-Norovirus GII.4 VP1 Calibrator 1 U/ml	0.65 ml	NG2002B
Anti-Norovirus GII.4 VP1 Calibrator 2.5 U/ml	0.65 ml	NG2002C
Anti-Norovirus GII.4 VP1 Calibrator 5 U/ml	0.65 ml	NG2002D
Anti-Norovirus GII.4 VP1 Calibrator 10 U/ml	0.65 ml	NG2002E
Anti-Human IgM HRP Conjugate (100X)	0.15 ml	H-HuM.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	NG2-HIM-055