AstraArt Norovirus qPCR Kit

PRINCIPLE

AstraArt Norovirus qPCR kit is a one-step real-time reverse transcriptase PCR for the simultaneous, qualitative detection of Norovirus genogroups I (GI) and II (GII) genotypes. The assay is a combination of the latest advanced buffer chemistry, PCR enhancers and stabilizers along with antibody-mediated hot-start polymerase, dNTPs and MgCl2. This assay has been designed for highly reproducible, accurate results in the presence of inhibitors, making it ideal for detection of lowest copy numbers of the target genes.

Norovirus virus is highly contagious. Only a low amount of virus particles is necessary for infection, leading to gastrointestinal disease. Thus, AstraArt Norovirus qPCR kit was designed for a high level of sensitivity with consistent specificity to detect the target genes in various food and water samples. The Norovirus primer and probe set(s) for VP1 Gene are designed to detect specific sequences of **GI** and **GII genotypes** along with **internal control.**

INSTRUCTIONS FOR USE

Avoid repeated freeze-thaw of reagents.

PACKAGE CONTENTS

Description	Specification	Quantity for 100 tests
qPCR Master Mix	qPCR Amplification Mix	1000 µL x 1 tube
Primer Mix	Target Specific Primer	500µL x 1 tube
Extraction Control (EC)	Synthetic gene	150 µL x 1 tube
Positive Control	Positive control	100µL x 1 tube
Negative control	Nuclease Free water	100 µL x 1 tube

Catalogue Number	Description
AG/NORO/23/01	Applicable for Norovirus testing

STORAGE &STABILITY

- All the reagents should be stored at - 20℃ (±5). Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. Avoid repeated freeze/thaw cycles for reagents.

SAMPLE REQUIREMENTS

- All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity.

ASSAY PROCEDURE:

Nucleic acid along with EC (1.5$\mu\ell$ per sample) is isolated from the sample using Nucleic acid extraction system. The purified nucleic acid is directly amplified using the AstraArt Norovirus qPCR kit on the Real-time PCR Instrument system. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Real-time PCR Instrument system.

a. Preparation of Real-time PCR reagents: Briefly Centrifuge all the reagents. Prepare the reagents according to the table below. The final volume is calculated by multiplying the number of samples by the volume of each component in Table 1.

Table 1: Components of reaction mix:

Component	Volume (µL) per reaction
qPCR Master Mix	10
Primer Mix	5
Template RNA/ Positive control/ Negative control	10
Total Volume	**25**

b. Mix the reaction mix except the template, and spin-down briefly. Aliquot 15 µL of the reaction mix into each well of 96-well plate and add 5µL of the template RNA/Positive control/Negative control accordingly. Seal the plate and spin-down briefly. Run the Protocol immediately on the Real-time PCR instrument with following cycling conditions in Table 2.

Table 2: Cycling Conditions:

Steps	Temperature° C	Time	Cycle
1 (cDNA Synthesis)	50	3 minutes	1
2 (Initial Denaturation)	95	2 minutes	1
3 (PCR Cycling)	95	05 seconds	40x
	60*	25 seconds	
IC uses Cy5, Noro GI gene uses TAMRA channel, Noro GII gene uses FAM channel, (*) Fluorescence is measured at 60° C.			

Note: Please select "None" in both Passive reference and Quencher. Depending on the Ramp rate of the PCR machine used the run times may vary.

c. Interpretation of Results:

Interpret the values for unknown samples based on the observations as described in the following table. There should be no amplification signal in negative control. ≤38 Ct of unknown samples should be considered for result interpretation.

Table 3: Conclusion:

	EC (Cy5)	GI (TAMRA)	GII (FAM)	Conclusion
***Positive Control**	-	+	+	Valid
***Negative Control**	-	-	-	Valid
Sample	+	+	-	Positive for Norovirus GI
Sample	+	-	+	Positive for Norovirus GII
Sample	+/-	+	+	Positive for GI and GII Norovirus
Sample	+	-	-	Negative
Sample	-	-	-	In conclusive repeat the PCR with fresh sample

LIMITATION OF THE PROCEDURE:

1. This kit is used for qualitative detection of Norovirus Genes from specimens. The results do not reflect the viral load in the original specimen.
2. The specimen to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
3. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure.
4. Amplification and detection of Norovirus Genotype with AstraArt Norovirus qPCR kit has only been validated with Real-Time PCR instruments.

WARNING & PRECAUTIONS:

- Do not use the product if there is evidence of leakage.
- Adhere to standard procedures and published protocols for sample collection, processing, and disposal.

SYMBOLS:

 Do not use if package is damaged  Manufacturer  Batch Code

 CE mark of Conformity  Refer to the instructions  ISO  GMP

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