# AstraArt Norovirus/Hepatitis A qPCR Kit

## PRINCIPLE

AstraArt Norovirus/ Hepatitis A qPCR kit is a one-step real-time reverse transcriptase PCR for the simultaneous, qualitative detection of Norovirus genogroups I (GI) and II (GII), Hepatitis A virus 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 and hepatitis A virus are highly contagious. Only a low amount of virus particles is necessary for infection, leading to either gastrointestinal disease (in case of norovirus) or liver infection (in the case of hepatitis A virus). Thus, AstraArt Norovirus & Hepatitis 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) are designed to detect specific sequences of **GI** and **GII genotypes and Hepatitis A to detect Genotypes of Hepatitis A virus** 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 | 150ul 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/NOHA/23/01 | Applicable for Norovirus and Hepatitis A 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/ Hepatitis A qPCR kit on the Real-time PCR Instrument system. In the process, the probe anneals 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 for qPCR | 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 10µL 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* | 30 seconds | |
| **IC uses Cy5, Noro GI gene uses TAMRA channel, Noro GII gene uses FAM channel, Hep A uses TxRd 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) | Hep A (TxRd) | 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** | + | - | - | + | Positive for Hepatitis A |
| **Sample** | +/- | + | - | + | Positive for Norovirus GI & Hepatitis A |
| **Sample** | +/- | - | + | + | Positive for Norovirus GII & Hepatitis A |
| **Sample** | +/- | + | + | + | Positive for Norovirus & Hepatitis A |
| **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 and Hepatitis A 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 and Hepatitis A Genes with AstraArt Norovirus/ Hepatitis A 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

AstraGene FZ LLC
Office No. 208 – 209, Dubai Science Park Building, Dubai, United Arab Emirates
+971-4-8781222, contact@astragene.com