Instructions for use
InviScreen® Norovirus Detection Kit

INVITEK diagnostics

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REF 6015002200 | Σ 100 tests | -25 °C / -15 °C | RUO

1. Intended use

InviScreen® Norovirus Detection Kit is designed for the qualitative detection of Norovirus genogroups GI and GII in food samples. This kit is intended for use by trained laboratory professionals in accordance with the ISO/TS 15216 standard. The PCR kit provides a sensitive and specific method for the detection of Norovirus genogroups GI and GII in various food matrices, including but not limited to shellfish, fruits, vegetables, and other ready-to-eat foods. Laboratory personnel should carefully follow the instructions provided in this manual, including the recommended sample preparation procedures, PCR protocol, and result interpretation guidelines. It is essential to adhere to good laboratory practices to minimize the risk of contamination and ensure accurate and reliable results.

2. Product description

InviScreen® Norovirus Detection Kit follows the standardised ISO/TS 15216 method that describes the recommendations for the detection of norovirus genogroups GI and GII. The method uses the real-time RT-PCR technique, following the ISO/TS 15216 specifications, to detect specific regions in the well-conserved 5′ end of the ORF1-ORF2 junction. The method is applicable to test samples of foodstuffs (soft fruit, leaf, stem and bulb vegetables, bottled water, bivalve molluscan shellfish) or surfaces. Amplification detection is based on Taqman technology, i.e., the fluorescence issued is detected upon degradation of the hydrolysis probe during PCR reaction. This kit includes a process control virus (virus to be added to the sample portion at the earliest opportunity prior to virus extraction), to control for extraction efficiency, and its respective primer and probe sets. The detection of amplified DNA fragments is achieved using different fluorescence channels (FAM, HEX and Cy5) available in common real-time thermocyclers.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D26.01	Primer/Probe Mix[1]	Targeted detection		1 tube, 250 µL
D26.02	RT-qPCR Master Mix (2x)[1]	Reverse Transcription Amplification		1 tube, 1250 µL
D26.03	DNase/RNase Free Water	Negative Control		1 tube, 1000 µL
D26.04	Positive Control	Positive Control		1 tube, 100 µL
D26.05	Process Control Virus	Extraction Control		4 tubes, 250 µL

[1] Reagents are supplied with a 5% of extra volume

4. Storage

Reagents should be stored sealed at -20 ± 5°C and may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles. Protect reagents from light exposure to prevent degradation.

5. Equipment and materials required (Not provided)

- Food processor [2]
- RNA extraction kit
- Real-Time PCR instrument [3]
- Plates and/or tubes for qPCR
- 1.5 mL microcentrifuge tubes
- PCR cabinet [4]
- Micropipettes (10, 200 and 1000 µL) and filter tips
- Vortex and microcentrifuge

[2] To ensure accurate analysis of samples with multiple ingredients that are not in a powdered or granulated form, it is recommended to homogenize them carefully.

[3] The assay was validated on Bio-Rad CFX96 and Agilent AriaMX instruments.

[4] To minimize the risk of contamination with foreign DNA, we recommend that the PCR reaction set-up is performed in a controlled environment

6. Suitable test sample material

Test samples of soft fruit, leaf, stem and bulb vegetables, bottled water, bivalve molluscan shellfish and environmental swabs can be used.

7. Test Procedure

a. Sample Preparation / RNA extraction

Losses of target virus can occur at several stages during sample virus extraction and RNA extraction. To account for these losses, samples must be spiked at the earliest opportunity prior to virus extraction with 10 µl of process control virus.

For water samples preparation, add 10 µl of process control virus to the water sample before centrifugation and/or filtration. For foodstuff samples, add 10 µL of process control virus to coarsely chopped samples. For environmental swab samples add 10 µl of process control virus after swab emulsion in lysis buffer. At the same time, it is highly recommended to prepare a process blank by adding 10 µL of Iprocess control virus to a norovirus-free water or food sample and process along with the samples to be analysed. Prepare the test sample in accordance with the specific International Standard ISO/TS 15216 considering the requirements for the specific product concerned. RNA should be extracted using any suitable method or commercially available kit. Extracted RNA shall be processed immediately or stored at 5 °C for a maximum of 24 h, at −15 °C or below for up to 6 months, or at −70 °C or below for longer periods.

b. PCR Reaction Preparation

Allow all reagents to thaw at room temperature, vortex and spin briefly to avoid drops on the vial cap. For each RNA sample prepare the reaction mix according to the table below:

REAGENT	VOLUME
RT-qPCR Master Mix	12.5 µL
Primer/Probe Mix	2.5 µL
DNase/RNase Free Water	5 µL
Total Volume	**20 µL**

1. Homogenize the reaction mixture and pipette 20 µL into individual wells according to the predicted PCR plate set-up.
2. Add 5 µL of RNA template to each well.

At least one positive control reaction and one negative control reaction must be included in the PCR run, replacing the sample in these wells with 5 µL of Positive Control and 5 µL of Negative Control, respectively.

It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone. The addition of the positive control and sample RNA should preferably be carried out in a separate room.

c. Amplification Protocol

The amplification conditions are as follows:

	STEPS	TEMPERATURE	TIME	CYCLES
1	Reverse Transcription	50 °C	20 min	1
2	Enzymatic Activation	95 °C	5 min	1
3	Denaturation	95 °C	15 sec	45
4	Hybridization/extension	60 °C	1 min	
5	Hybridization/extension plate reading *	65 °C	1 min	

*Fluorescence data must be obtained during this step through FAM, HEX and Cy5 channels.

d. Results Interpretation

The results should be interpreted in accordance with the analysis software recommended by the Real-Time PCR instrument manufacturer. The software monitors cDNA amplification through the detection of fluorescence emitted by each probe, attributing a Ct value for each reporter dye found in each individual sample. Norovirus Target DNA amplification is monitored in HEX (GI) and Cy5 (GII). Amplification of process control virus is monitored in FAM. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred bellow.

A result is considered positive when Ct ≤ 45.

RESULT	Target RNA (HEX/Cy5)	Process Control Virus (FAM)
Positive	+	+/-
Negative	-	+
Inconclusive [5,6]	-/?	-

[5] PCR inhibitions may be due to the presence of excessive RNA and/or PCR inhibitors. It is recommended to dilute the RNA extracted from the sample 1:10 in DNAse/RNAse free water and repeat the Real-Time PCR reaction. When applicable, the LOD ofthe method should be adjusted in accordance with the dilution factor.
[6] The appearance and characteristics of the amplification curves should be thoroughly considered. Incomplete amplification curves often denote low amount of RNA template. In this case, the positivity of the result is dubious, and the Real-Time PCR reaction should be repeated using a superior amount of RNA template.

e. Expression of results

Samples negative for GI and/or GII: GI and/or GII virus genome not detected in the test portion of x g or x mL or x cm2.

Samples positive for GI and/or GII: GI and/or GII virus genome detected in the test portion of x g or x mL or x cm2.

8. Quality Control

The test can only be considered valid under the following control conditions:

CONTROLS	Target DNA	Process Control Virus
Positive Control	+	+
Negative Control	-	-
Process Blank [7]	-	+

[7] Highly recommended

If no amplification is observed for the positive control, the test results are invalid and must be repeated. The positive control and process blank are expected to amplify before Ct 35.
If amplification is observed for the negative control, it indicates that the reagents have become contaminated while setting up the run, invalidating test results.

9. Performance Characteristics

Specificity: The specificity of the kit is first and foremost ensured through the selection of primers and hydrolysis probes, as well as optimization of reaction conditions. Additionally, method specificity was evaluated using internal reference materials, spiked samples and specimens received in the laboratory for analysis and identified by other techniques. The list of internal reference materials includes a group of viruses and bacteria (not detected by the kit). None of the pathogens tested with the kit were cross-reactive, such as Porcine reproductive and respiratory syndrome (Leystad Virus - EU strain), Porcine reproductive and respiratory syndrome (VR-2332 - US strain), Infectious Bursal disease Virus (D78 strain), Avian Reovirus, *Salmonella enteritidis, Salmonella typhimurium, Listeria monocytogenes, Vibrio vulnificus, Vibrio parahaemolyticus*, and *Staphylococcus aureus*.

Analytical Sensitivity and Limit of Detection: The Limit of detection (LOD) of the method was determined using positive reference material containing the genes of interest. The reference material was adjusted to $1x10^6$ copies/µL using nuclease free water and serial diluted to 1 copy/µL of GI and GII. Under optimal conditions, the lowest amount of norovirus RNA detected in 100% of the experiments was at least 10 copies/µL. Different samples were spiked with known concentrations of norovirus GI and GII and with the respective process control virus sample ($1x10^5$ copies/µL). The LOD is often matrix dependent, and the sensitivity of the analysis may be reduced depending on the total RNA extracted, but also its quality.