

foodproof®

Norovirus (GI, GII) plus Hepatitis A Virus Detection Kit

Ready Reference Guide

Revision A, December 2023

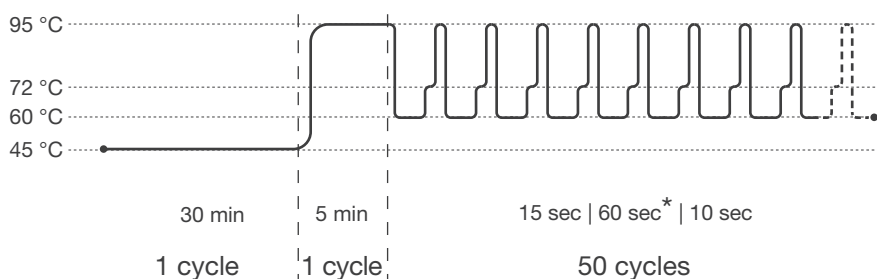
Product No. KIT230056

PCR kit for the qualitative detection of hepatitis A virus and norovirus genogroup I and II using real-time PCR instruments. Before starting, it is strongly recommended to read the entire product manual available on our website.

PROGRAM SETUP

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- FAM (hepatitis A virus), HEX (norovirus GI), ROX (norovirus GII) and Cy5 (Process Control).



Reverse transcription: 1 cycle

Step 1: 45 °C for 30 min

Initial denaturation: 1 cycle

Step 1: 95 °C for 5 min

Amplification 50: cycles

Step 1 : 95 °C for 15 sec

Step 2*: 60 °C for 60 sec

Step 3 : 72 °C for 10 sec

* Fluorescence detection

For some real-time PCR instruments the probe quencher as well as the usage of a passive reference dye has to be specified. This kit contains probes with a non-fluorescent "dark" quencher and no passive reference dye. For the LightCycler® 480 System, generation of a color compensation data file is necessary. Please use Color Compensation Set 3 (Product No. KIT230005).

DATA INTERPRETATION

Verify results of positive (Control Template) and negative controls (H₂O), before interpreting sample results. Always compare samples to positive and negative control. Review data from each channel and interpret results as described in the table.

FAM	HEX	ROX	Cy5	Result Interpretation
+	+	+	+ or -	Positive for hepatitis A virus, norovirus GI, norovirus GII
-	+	+	+ or -	Positive for norovirus GI, norovirus GII
+	-	+	+ or -	Positive for hepatitis A virus, norovirus GII
+	+	-	+ or -	Positive for hepatitis A virus, norovirus GI
-	+	-	+ or -	Positive for norovirus GI
+	-	-	+ or -	Positive for hepatitis A virus
-	-	+	+ or -	Positive for norovirus GII
-	-	-	+	Negative for hepatitis A virus, norovirus GI, norovirus GII
-	-	-	-	Invalid

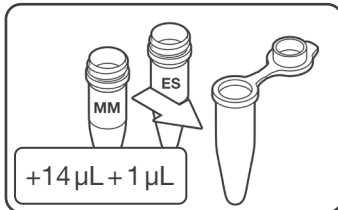
PREPARATION OF THE RT-PCR MIX

Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.

Thaw reagents, mix (do not vortex!), and briefly spin vials before opening.

Reverse transcriptase is a very temperature-sensitive enzyme. It is recommended to use a cooling block (at 4 °C) to avoid degradation of the Enzyme Solution (red cap).

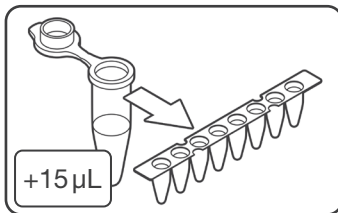
After usage, store the enzyme solution immediately at -15 to -25 °C.



1. PREPARE RT-PCR MIX

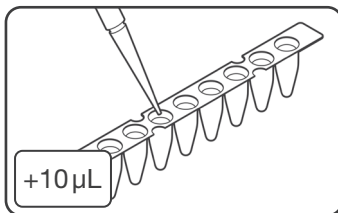
Add 14 µL of Master Mix (yellow cap) and 1 µL Enzyme Solution (red cap) for each reaction to a suitable tube (n samples + 2 controls + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



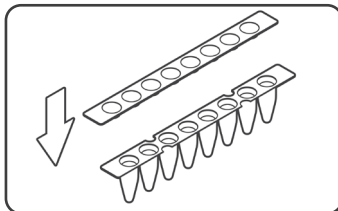
2. ADD RT-PCR MIX

Pipet 15 µL of prepared RT-PCR mix into each strip or plate well.



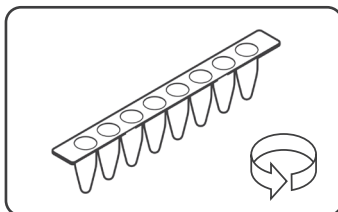
3. ADD SAMPLES AND CONTROLS

Pipet 10 µL of samples, Negative Control (orange cap) or Control Template (purple cap) into respective wells.



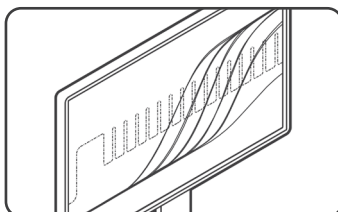
4. SEAL

Seal strips/plate accurately.



5. CENTRIFUGE

Briefly spin strips/plate in a suitable centrifuge.



6. START REAL-TIME PCR RUN

Cycle samples as described above.