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# Hepatitis A Virus Detection Kit

## Ready Reference Guide

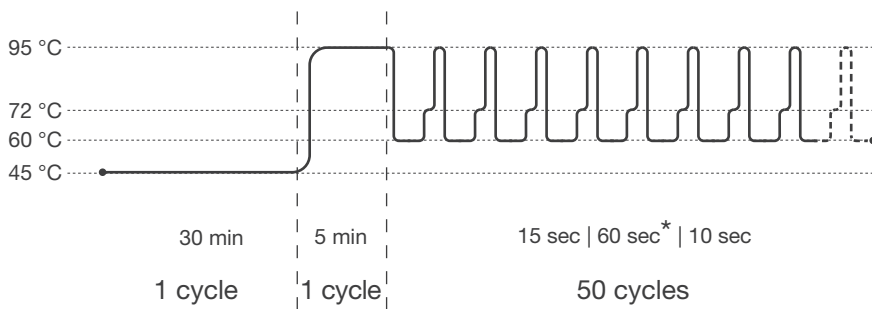
Revision A, December 2023

Product No. KIT230054

PCR kit for the qualitative detection of hepatitis A virus 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) and ROX (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.

### DATA INTERPRETATION

Verify results of positive (Control Template) and negative controls (H<sub>2</sub>O), 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.

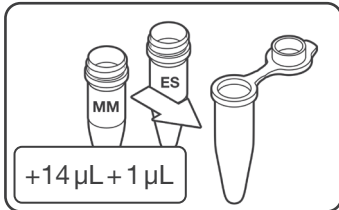
FAM	ROX	Result Interpretation
+	+ or -	Positive for hepatitis A virus
-	+	Negative for hepatitis A virus
-	-	Invalid

# 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).

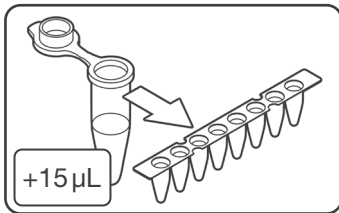
After usage, store the enzyme solution immediately at -15 to -25 °C.



## 1. PREPARE RT-PCR MIX

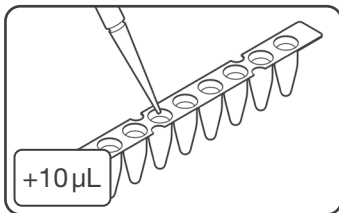
Add 14 µL of Master Mix (yellow cap) and 1 µL Enzyme Solution (red cap) for each reaction to a suitable tube (n samples + 2 controls + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



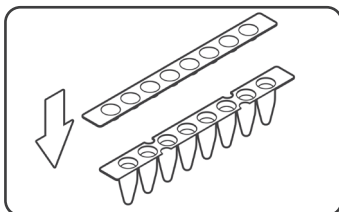
## 2. ADD RT-PCR MIX

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



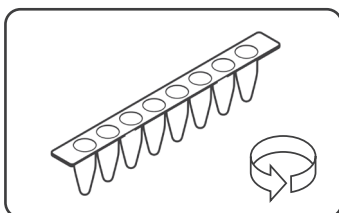
## 3. ADD SAMPLES AND CONTROLS

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



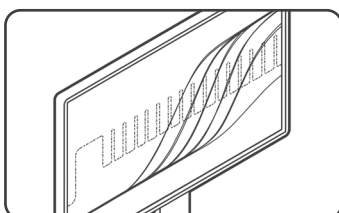
## 4. SEAL

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.