foodproof® Hepatitis A Virus Detection Kit

Revision A, December 2023

RT-PCR kit for the qualitative detection of hepatitis A virus and MS2-RNA using real-time instruments.

Product No. KIT230054

Kit for 64 reactions for a maximum of 62 samples

Store the kit at –15 to –25 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

Table of Contents

1. Product Overview

1.1 Number of Tests

The kit is designed for 64 reactions [Master Mix (vial 1, yellow cap)] with a final reaction volume of 25 µL each. Up to 62 samples plus one positive control [Control Template (vial 4, purple cap)] and one negative control [Negative Control (vial 5, orange cap)] reaction can be analyzed per run.

1.2 Storage and Stability

- Store the kit at –15 to –25 °C until the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following Kit Contents table.

1.3 Kit Contents

Vial	Label	Contents / Function / Storage
1 yellow cap	foodproof® Hepatitis A Virus Detection Kit Master Mix	• 2 x 500 µL • Ready-to-use primer and hydrolysis probe mix specific for hepatitis A virus and the Process Control / Internal Amplification Control • For amplification and detection of hepatitis A virus sequences • Store at –15 to –25 °C • **Avoid repeated freezing and thawing!** • **Protect from light!**
2 red cap	foodproof Hepatitis A Virus Detection Kit Enzyme Solution	• 2 x 40 µL • Contains Reverse Transcriptase and a yellow dye for better visualization • Store at –15 to –25 °C. A high-quality freezer is very important. • Reverse transcriptase is a very temperature-sensitive enzyme. After usage, store the enzyme solution immediately at –20 °C.
3 white cap	foodproof Hepatitis A Virus Detection Kit Process Control	• 3 x 250 µL • Contains a stabilized solution of MS2 phage • For use as preparation / internal amplification control • Added to the samples at the beginning of the protocol • Store at –15 to –25 °C • Only thaw RNA on ice or in a 4 °C cooling block! • **Avoid repeated freezing and thawing!**
4 purple cap	foodproof Hepatitis A Virus Detection Kit Control Template	• 1 x 140 µL • Contains a stabilized solution of DNA specific for hepatitis A virus and the Process Control • For use as run positive control with internal amplification control • Store at –15 to –25 °C • **Avoid repeated freezing and thawing!**

Vial	Label	Contents / Function / Storage
5 orange cap	foodproof Hepatitis A Virus Detection Kit Negative Control	• 1 x 140 µL • Contains a stabilized solution of DNA of the Process Control • For use as run negative control with internal amplification control • Store at –15 to –25 °C • **Avoid repeated freezing and thawing!**
6 colorless cap	foodproof Hepatitis A Virus Detection Kit H_2O, PCR-grade	• 3 x 1 mL • Nuclease-free, PCR-grade H_2O • For use as dilution reagent • After first thawing, store at 2 to 8 °C

1.4 Product Description

The foodproof Hepatitis A Virus Detection Kit is a one-step real-time reverse-transcription PCR for the simultaneous, qualitative detection of the hepatitis A virus RNA and a process control / internal amplification control providing a comprehensive and fast interpretation of the results. The kit includes primers and hydrolysis probes (for sequence-specific detection), convenient premixed reagents and a control template for reliable interpretations of results. The foodproof Hepatitis A Virus Detection Kit is based on primer, probes and methods that are mentioned in the ISO/TS 15216 [4].

The hepatitis A virus can cause a self-limiting liver infection and is highly contagious. Thus, the kit was designed for high-level sensitivity with consistent specificity. Besides transmission via person-to-person and the fecal-oral-route, the virus can also be transmitted by food and water. The foodproof Hepatitis A Virus Detection Kit was designed and validated for use in food diagnostics.

To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification by diverse sample matrices (soft fruits, minced meat, water, shellfish), the Process Control (vial 3, white cap, contains MS2 phage) must be added to the examined sample at the beginning of sample processing. The viral RNA must be extracted by the foodproof Sample Preparation Kit IV (Product No. KIT230185) and can subsequently be analyzed by the foodproof Hepatitis A Virus Detection Kit.

The same already-transcribed RNA (now cDNA) of this preparation control is already added as the “Internal Amplification Control” to the Negative Control (vial 5, orange cap) and the Control Template (vial 4, purple cap). The hydrolysis probe was designed to bind specifically to the Process Control, allowing detection in the ROX channel, whereas the hepatitis A viral RNA is detected in the FAM channel.

Note: *More detailed information is listed in the* Validation Data Report *of the foodproof Hepatitis A Virus Detection Kit. Please contact our Technical Support at www.hygiena.com/support.*

1.5 Application

The foodproof Hepatitis A Virus Detection Kit is intended for food testing purposes. It is used to identify purified hepatitis A viral RNA prepared and purified by the foodproof Sample Preparation Kit IV (Product No. KIT230185).

1.6 Background Information

Hepatitis A virus is a small, non-enveloped virus and a member of the *Picornaviridae* family. The genome is a single, positive-stranded RNA. Currently, the virus is divided into seven genotypes, whereby only genotypes I – III are known to infect humans [2]. The most common genotype is I (Ia more common than Ib) and is distributed worldwide. It can cause a liver infection but also often causes mild or asymptomatic disease. Several food-related outbreaks of hepatitis A virus have already been reported [1, 3]. The potential food matrices are linked to those of noroviruses. Therefore, the same analytical methods can be used for both viruses [4]. Since the PCR thermal profile of the foodproof Hepatitis A Virus Detection Kit is identical to the profile of the foodproof Norovirus Detection Kit (KIT230055), both kits can be used in the same run. Since cell culture methods are time-consuming and not very sensitive, the reverse transcriptase-polymerase chain reaction has become the method of choice and is used as the gold-standard [4].

2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Detection of hepatitis A viral RNA using the foodproof Hepatitis A Virus Detection Kit requires RNA transcription to DNA and DNA amplification by PCR.

The kit provides all reagents required for reverse transcription and real-time PCR. However, to achieve reliable results, the entire procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carryover-, or cross-contamination:

- Prepare appropriate aliquots of the kit solutions and keep them separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear powder-free gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use sterile aerosol-preventive pipette tips.
- To avoid carryover contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- Physically separate the workplaces for RNA preparation, PCR setup and PCR to minimize the risk of carryover contamination. Use a PCR hood for all pipetting steps.

Note*:* Protect the Master Mix (vial 1, yellow cap) from light and avoid multiple freezing and thawing cycles.

2.1.2 Additional Equipment and Reagents Required

- Real-time PCR instrument suitable for detection of FAM-, VIC/HEX-, and ROX-labeled probes
- Real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR cycler in use
- foodproof Sample Preparation Kit IV (Product No. KIT230185)
- Pipettes
- Nuclease-free, aerosol-resistant pipette tips
- Sterile reaction tubes for preparing PCR mixes and dilutions
- Powder-free gloves

2.1.3 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors. For preparation of genomic RNA from raw material, refer to the corresponding product package inserts of a suitable sample preparation kit (see *Additional Equipment and Reagents Required* above).

2.1.4 Assay Time

Procedure	Time
PCR Setup	15 min
PCR run	140 min (e.g., LC 480 II)
Total assay time	155 min

2.1.5 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template RNA with the provided control DNA [foodproof Hepatitis A Virus Detection Kit - Control Template (vial 4, purple cap)] or with a positive sample preparation control.

2.1.6 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template RNA with the provided no template control foodproof Hepatitis A Virus Detection Kit – Negative Control (vial 5, orange cap)]. Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.1.7 Process Control

Always run the Process Control (vial 3, white cap) together with the samples. To prepare a Process Control, pipet 10 µL of the control virus to the sample at the first step of the RNA preparation procedure [see foodproof Sample Preparation Kit IV, Product No. KIT230185]. For some sample matrices, a virus pre-concentration step is necessary (e.g., for soft fruits and food vegetables, bottled water and bivalve molluscan shellfish; ISO/TS 15216). For that, the Process Control has to be added at the start of sample processing.

2.2 Program Setup

Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time-program (for details on how to program the experimental protocol, see the operation manual of your real-time PCR cycler):

Reverse transcription	**1** cycle:	Step 1: 45 °C for 30 minutes
Pre-incubation	**1** cycle:	Step 1: 95 °C for 5 minutes
Amplification	**50** cycles:	Step 1: 95 °C for 15 seconds Step 2*: 60 °C for 60 seconds Step 3: 72 °C for 10 seconds

*Flourescence detection in step 2

Note: For some real-time PCR instruments (e.g., ABI 7500) the type of the probe quencher as well as the usage of a passive reference dye must be determined. The foodproof Hepatitis A Virus Detection Kit contains probes with a non-fluorescent quencher and no passive reference dye.

For users of the Agilent Mx3005p instrument: Click "Instrument Filter Set Gain Settings" to open the Filter Set Gain Settings dialog box. For FAM, the Filter Set Gain Setting must be modified to "x4".

2.3 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction.

Always wear gloves when handling the PCR vessels.

1. Completely thaw the foodproof Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) and Enzyme Solution (vial 2, red cap) at room temperature (~25 °C). For maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully but thoroughly by pipetting up and down.
2. In a reaction tube (0.5 – 2.0 mL, depending on the number of reactions), prepare the PCR mix by adding the following components in the order mentioned below, then mix gently but thoroughly by pipetting up and down.

   The volumes indicated below are based on a single 25 µL standard reaction. Prepare the PCR mix by multiplying the amount in the "Volume" column by the number of reactions (sample and control reactions) to be cycled plus one or two additional reactions to cover pipetting losses.

Component, foodproof Hepatitis A Virus Detection Kit	Volume
Master Mix , (vial 1, yellow cap)	14 µL
Enzyme Solution, (vial 2, red cap)	1 µL
Total volume	15 µL

3. Prepare the reaction mixtures:
   - Pipet 15 µL of PCR mix into each PCR vessel.
   - For the samples of interest, add up to 10 µL sample RNA (if <10 µL add H2O (vial 6, colorless cap) to 10 µL). **Note**: Thaw RNA only on ice or in a 4 °C in a cooling block.
   - For the negative control, add 10 µL foodproof Hepatitis A Virus Detection Kit, Negative Control (vial 5, orange cap).
   - For the positive control, add 10 µL foodproof Hepatitis A Virus Detection Kit, Control Template (vial 4, purple cap).
4. Seal the PCR vessels accurately with optical caps or sealing foil.
5. Briefly spin the PCR vessels in a suitable centrifuge.
6. Cycle the samples as described above.

2.4 Data Interpretation

The amplification of hepatitis A Viral RNA is analyzed in the fluorescence channel suitable for FAM-labeled probes. The specific amplification of the Process Control is analyzed in the fluorescence channel suitable for ROX. Compare the results from the FAM channel (hepatitis A virus) and ROX channel (Process Control) for each sample, and interpret the results as described in the table below.

Hepatitis A Virus FAM Channel	Process Control ROX Channel	Result Interpretation
Positive	Positive / Negative	Positive for hepatitis A virus
Negative	Positive	Negative for hepatitis A virus
Negative	Negative	Invalid

Note*:* A prerequisite for the unambiguous detection of the hepatitis A virus as well as Process Control RNA in this multi-color experiment is a suitable calibration of the PCR Instrument for FAM and ROX channels. Please refer to the operation manual of your real-time PCR cycler for further information. A Color Compensation (Color Compensation Set 3; Product No: KIT230005) is necessary and will be supplied by Hygiena Diagnostics for users of the LightCycler 480 Systems I and II. Please contact Hygiena Diagnostics for further information.

Check the results for each control and compare the results with the expected results as described in the table below.

Hepatitis A Virus FAM Channel	Process Control ROX Channel	Expected results
Positive	Positive	foodproof Hepatitis A Virus Detection Kit Control Template
Negative	Positive	foodproof Hepatitis A Virus Detection Kit Negative Control
Negative	Positive	Process Control after sample processing and RNA extraction
Negative	Negative	foodproof Hepatitis A Virus Detection Kit H_2O, PCR-grade

3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	Set Channel settings to FAM and ROX
	Pipetting errors or omitted reagents.	• Check for correct pipetting scheme and reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	Check the cycle programs.
No signal increase is observed, even with the Process control (ROX).	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Use the recommended RNA sample preparation kit to purify template RNA. • Dilute samples 1 to 10 (e.g., 1 µL sample to 9 µL H_2O).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) at –15 to –25 °C, protected from light. • Avoid repeated freezing and thawing**.**
	foodproof Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) is not homogeneously mixed.	• Mix the Master Mix (vial 1, yellow cap) and the entire PCR-mix thoroughly before pipetting.
	Low initial amount of target RNA.	Increase the amount of sample RNA. Depending on the chosen RNA isolation method, inhibitory effects may occur.
Negative control samples are positive.	Carryover contamination.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carryover contamination. • Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR vessels. Prepared PCR mix is still in the upper part of the vessel.	Always centrifuge reaction vessels.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	Always wear powder-free gloves when handling the vessels and seal.
Precipitation of the foodproof Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap)	Incomplete thawing of the Master Mix (vial 1, yellow cap)	Warm up the Master Mix carefully in your hands and tap the tube gently until the precipitation gone (do not vortex!).
	Precipitation of stabilizing reagents in the Master Mix (vial 1, yellow cap)	

4. References

1. European Food Safety Authority. (2014) Tracing of Food Items in Connection to the Multinational Hepatitis A Virus Outbreak in Europe. EFSA Journal 12(9):3821. http://doi.org/10.2903/j.efsa.2014.3821
2. Costa-Mattioli M, Napoli AD, Ferre V, Billaudel S, Perez-Bercoff R and Cristina J. (2003) Genetic Variability of Hepatitis A Virus. J Gen Virol, 84(12), 3191 – 3201. http://doi.org/10.1099/vir.0.19532-0
3. Fiore AE. (2004). Hepatitis A Transmitted by Food. Clin Infect Dis, 38(5):705 – 715. http://doi.org/10.1086/381671
4. Microbiology of food and animal feed - Horizontal Method for Determination of Hepatitis A Virus and Norovirus in Food Using Real-Time RT-PCR – Part 2: Method for Qualitative Detection (ISO/TS 15216-2:2013); German version CEN ISO/TS 15216-2:2013.

5. Supplementary Information

5.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

5.2 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Hygiena® is a trademark of Hygiena.

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5.3 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. Contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

5.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 302 37

6. Change Index

Version 1, June 2017
First version of the manual.

Version 2, September 2017
License Notice removed.

Revision A, December 2023
Rebranding and new layout.
R 302 37 20 -> INS-KIT230054-REVA

Hygiena®
Camarillo, CA 93012
USA
diagnostics.support@hygiena.com

Manufactured by
Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam
Germany
www.hygiena.com