For food testing purposes

FOR *IN VITRO* USE ONLY

foodproof® Hepatitis A Virus Detection Kit – 5´Nuclease –

Version 2, September 2017

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

Order No. R 302 37

Kit for 64 reactions for a maximum of 62 samples

Store the kit at -15 to -25 °C

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1. Product Overview

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.

Storage and Stability

- Store the kit at -15 °C 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

Kit Contents

Vial	Label	Contents / Function / Storage
1 yellow cap	**food**proof® 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	**food**proof® 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. Good-working 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	**food**proof® 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 • Store at -15 to -25 °C. • Only thaw RNA on ice or at 4 °C cooling block! • **Avoid repeated freezing and thawing!**
4 purple cap	**food**proof® Hepatitis A Virus Detection Kit Control Template	• 1 x 140 µl • Contains a stabilized solution of DNA specific for hepatitis A virus and Process Control. • For use as run positive control with internal amplification control. • Store at -15 to -25 °C. • **Avoid repeated freezing and thawing!**
5 orange cap	**food**proof® 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	**food**proof® 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 °C to +8 °C.

Product Description

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

The hepatitis A Virus can cause a self-limiting liver infection. The hepatitis A virus is highly contagious. Thus, the kit was designed for high level on sensitivity with consistent specificity. Beside the transmission via person-to-person and the fecal-oral-route, the virus can also be transmitted by food and water. The **food**proof® Hepatitis A Virus Detection Kit was designed and validated for the use in food diagnostic.
To ensure maximum reliability of the kit and to prevent misinterpretation of negative results, due to inhibition of the amplification by divers sample matrices (soft fruits, minced meat, water, shellfish), the Process Control (vial 3, white cap, contains MS2 phage) has to be added to the examined sample at the beginning of sample processing. The viral RNA has to be extracted by the **food**proof® Sample Preparation Kit IV (S 400 16) and can subsequently analysed by the **food**proof® Hepatitis A Virus Detection Kit.

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

Note: *More detailed information is listed in the Validation Data Report of the* ***food****proof Hepatitis A Virus Detection Kit. Please contact our Technical Support (bcd@bc-diagnostics.com).*

Application

The **food**proof® Hepatitis A Virus Detection Kit is intended for food testing purposes. It is used to identify purified hepatitis A virus RNA prepared and purified by the **food**proof® Sample Preparation Kit IV (S 400 16).

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 it is distributed worldwide. It can cause a liver infection, but it also often causes only 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, same analyzing methods can be used for both viruses [4]. Since the PCR thermal profile of the **food**proof® Hepatitis A Virus Detection Kit is identical of the profile of the **food**proof® Norovirus Detection Kit (R 302 38.1), both kits can be used within one 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

Precautions and Warnings

Detection of hepatitis A virus RNA using the **food**proof® Hepatitis A Virus Detection Kit requires RNA transcription to DNA and DNA amplification by PCR. The kit provides all reagents required for the reverse transcription and for real-time PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carry-over-, 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 carry-over 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 carry-over 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.

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
• **food**proof® Sample Preparation Kit IV [1] (Order No. S 400 16)
• Nuclease-free, aerosol-resistant pipette tips
• Pipettes
• Sterile reaction tubes for preparing PCR mixes and dilutions
• powder-free gloves

[1] Available from BIOTECON Diagnostics; see Ordering Information for details

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"*).

Assay Time

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

Positive Control

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

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 **food**proof® 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.

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 **food**proof® Sample Preparation Kit IV, S 400 16]. 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 to the start of the 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 has to be determined. The **food**proof® 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 in which the gain settings may be viewed and modified. 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 solutions **food**proof® 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	Volume
foodproof® Hepatitis A Virus Detection Kit, Master Mix , (vial 1, yellow cap)	14 µl
foodproof® Hepatitis A Virus Detection Kit, Enzyme Solution, (vial 2, red cap)	1 µl
Total volume	**15 µl**

3. • Pipet 15 µl PCR mix into each PCR vessel.
   • For the samples of interest, add up to 10 µl sample RNA (if less than 10 µl add H_2O (vial 6, colorless cap) to 10 µl).
   Note: Thaw RNA only on ice or at 4 °C in a cooling block
   • For the negative control, add 10 µl **food**proof® Hepatitis A Virus Detection Kit, Negative Control (vial 5, orange cap).
   • For the positive control, add 10 µl **food**proof® 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 the hepatitis A Virus 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 channel FAM (hepatitis A virus) and channel ROX (Process Control) for each sample, and interpret the results as described in the table below.

Hepatitis A Virus Channel FAM	**Process Control Channel ROX**	**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 channels FAM and ROX. Please refer to the operation manual of your real-time PCR cycler for further information. A Color Compensation (Color Compensation Set 3; Order No. A 500 10) is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 Systems I and II. Please contact BIOTECON 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 Channel FAM	**Process Control Channel ROX**	**Expected results**
Positive	Positive	**food**proof® Hepatitis A Virus Detection Kit Control Template
Negative	Positive	**food**proof® Hepatitis A Virus Detection Kit Negative Control
Negative	Positive	Process Control after sample processing and RNA extraction
Negative	Negative	**food**proof® 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 any channel even in ROX for the Process control is observed.	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 **food**proof® Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap) at -15 °C 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 **food**proof® Hepatitis A Virus Detection Kit, 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.	Carry-over 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 • Add positive controls after sample and negative control Reaction vessels have been sealed commonly accepted practices to prevent carry-over contamination.
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 **food**proof® Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap)	Incomplete thawing of the Master Mix the **food**proof® Hepatitis A Virus Detection Kit, Master Mix (vial 1, yellow cap)	• Warm up the Master Mix carefully in your hands and snap gently to the tube until the precipitation gone (do not vortex!)
	Precipitation of stabilizing reagents in the **food**proof® Hepatitis A Virus Detection Kit, 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 2014;12(9):3821, 12(9), 1–186. http://doi.org/10.2903/j.efsa.2014.3821

2. Costa-Mattioli, M. (2003). **Genetic variability of hepatitis A virus**. Journal of General Virology, 84(12), 3191–3201. http://doi.org/10.1099/vir.0.19532-0

3. Fiore, A. E. (2004). **Hepatitis A transmitted by food**. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 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

BIOTECON Diagnostics is offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com (order@bc-diagnostics.com).

5.2 Trademarks

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Other brand or product names are trademarks of their respective holders.

5.3 Contact and Support

If you have questions about this or any other product of BIOTECON Diagnostics, please contact our technical support staff (bcd@bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective support. We also want you to 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.

6. Change Index

Version 1, June 2017:
First version of the manual.

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License Notice removed.

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