altona DIAGNOSTICS

Instructions for Use

AltoStar®
HAV RT-PCR Kit 1.5

02/2021 EN

AltoStar®

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HAV RT-PCR Kit 1.5

For research use only!

(RUO)

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altona Diagnostics GmbH • Mörkenstr. 12 • D-22767 Hamburg

Content

1. Application

The AltoStar® HAV RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of hepatits A virus (HAV) specific RNA.

For research use only (RUO)! Not for use in diagnostic procedures.

2. Product Content

The AltoStar® HAV RT-PCR Kit 1.5 contains the following components:

Lid Color	AltoStar® HAV 1.5 Component	Number of Tubes	Nominal Volume [µl/Tube]
Blue	Master A	8	60
Purple	Master B	8	180
Red	PC*	2	250
White	NTC**	2	250

* Positive Control (HAV)

** No Template Control

3. Storage

- The AltoStar® HAV RT-PCR Kit 1.5 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored at -25 °C to -15 °C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay.
- Storage at +2 °C to +8 °C should not exceed a period of 2 hours.
- Protect Master A and Master B from light.

4. Product Description

The AltoStar® HAV RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of hepatits A virus specific RNA.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labeled with fluorescent reporter and quencher dyes.

In addition to the HAV RNA specific amplification and detection systems the AltoStar® HAV RT-PCR Kit 1.5 includes oligonucleotides for the amplification and detection of an Internal Control (IC, AltoStar® Internal Control 1.5). For details refer to the Instructions for Use of the AltoStar® Internal Control 1.5.

Probes specific for HAV are labeled with the fluorophore FAM™. The probe specific for the Internal Control is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of HAV and the IC in the corresponding detection channels of the real-time PCR instrument.

4.1 Components

The AltoStar® HAV RT-PCR Kit 1.5 contains enough reagents for 96 reactions. The product consists of the following components:

- Master A
- Master B
- PC*
- NTC**

* Positive Control (HAV)

** No Template Control

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of HAV specific RNA and the IC (AltoStar® Internal Control 1.5) in one reaction setup.

The Positive Control (PC) contains HAV specific RNA. It is used to verify the functionality of the HAV specific amplification and detection systems.

The No Template Control (NTC) contains no HAV specific RNA but does contain the IC template. The NTC is used as negative control for the HAV RNA specific real-time PCR and indicates possible contamination of Master A and Master B.

4.2 Real-Time PCR Instruments

The AltoStar® HAV RT-PCR Kit 1.5 can be used with the following real-time PCR instruments:

- CFX96™ Dx System (Bio-Rad)
- CFX96™ Deep Well Dx System (Bio-Rad)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

5. Material Required but not Provided

The following additional instruments and consumables are required for use of the AltoStar® HAV RT-PCR Kit 1.5 but not provided with this product:

- Appropriate real-time PCR instrument (see chapter 4.2 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 6.1 Sample Preparation)
- Vortex mixer
- Centrifuge (e.g. desktop centrifuge) for centrifugation of kit reagents
- Centrifuge for centrifugation of PCR plates
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

Reagents required but not included in the AltoStar® HAV RT-PCR Kit 1.5:

- AltoStar® Internal Control 1.5 (Order No. IC15-06)

6. Procedure

6.1 Sample Preparation

Extracted RNA is the starting material for the AltoStar® HAV RT-PCR Kit 1.5. The quality of the extracted RNA has a profound impact on the performance of the product.

For additional information and technical support regarding pre-treatment and sample preparation please contact our technical support (see chapter 9. Technical Assistance).

6.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The AltoStar® HAV RT-PCR Kit 1.5 is configured for use with the AltoStar® Internal Control 1.5 (IC), which allows to control the sample preparation procedure (nucleic acid extraction) and the subsequent RT-PCR.

- ► Add the IC during the lysis step of the nucleic acid extraction procedure.
- ► No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 50 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 30 µl of IC per sample must be added into the sample/lysis buffer mixture.
- ► Set up the master mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

NOTE

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

6.3 Reaction Setup

- ► Pipette 20 µl of the master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (PC or NTC).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that at least 1 PC and 1 NTC is used per run.
- ► Thoroughly mix the samples and controls with the master mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1 000 x g (~ 3 000 rpm).

NOTE

Do not add the IC to the PC reactions and the NTC.

7. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the AltoStar® HAV RT-PCR Kit 1.5 on specific real-time PCR instruments please contact our technical support (see chapter 9. Technical Assistance).

7.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

7.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
HAV specific RNA	HAV	FAM™	(None)
Internal Control	IC	JOE™	(None)

7.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	55	00:45
			-	72	00:15

8. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the AltoStar® HAV RT-PCR Kit 1.5 on different real-time PCR instruments please contact our technical support (see chapter 9. Technical Assistance).

8.1 Interpretation of Results

8.1.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™ (HAV)	JOE™ (IC)	
+	+/-*	HAV specific RNA detected.
-	+	No HAV specific RNA detected. Sample does not contain detectable amounts of HAV specific RNA.
-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the IC in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high target RNA load in the sample can lead to reduced or absent IC signal.

9. Technical Assistance

For customer support, please contact our technical support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

10. Trademarks and Disclaimers

AltoStar® (altona Diagnostics); CFX96™ (Bio-Rad); JOE™ (Life Technologies); FAM™, ROX™ (Thermo Fisher Scientific).

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For research use only (RUO)! Not for use in diagnostic procedures.

11. Explanation of Symbols

Symbol	Explanation
RUO	Research Use Only
LOT	Batch code
CONT	Content
CAP	Cap color
REF	Catalogue number
NUM	Number
COMP	Component
	Consult instructions for use
	Contains sufficient for "n" tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
MAT	Material number
	Version
	Note: Information is given to the user that is useful but not essential to the task at hand.

always a drop ahead.

altona Diagnostics GmbH
Mörkenstr. 12
22767 Hamburg, Germany

phone +49 40 548 0676 0
fax +49 40 548 0676 10
e-mail info@altona-diagnostics.com

www.altona-diagnostics.com