

Instructions for use

AltoStar[®] HBV PCR Kit 1.5

04/2023 EN

AltoStar[®]

HBV PCR Kit 1.5

For research use only!

(RUO)

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96



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1. Application

The AltoStar® HBV PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the detection and quantification of hepatitis B virus (HBV) specific DNA.

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

2. Kit content

The AltoStar® HBV PCR Kit 1.5 contains the following components:

Table 1: Kit components

Lid color	Component	Number of tubes	Nominal volume [µl/tube]
Blue	Master A ¹⁾	8	240
Purple	Master B ¹⁾	8	300
Red	QS1 ²⁾	2	550
Red	QS2 ²⁾	2	550
Red	QS3 ²⁾	2	550
Red	QS4 ²⁾	2	550
White	NTC ³⁾	2	550

¹⁾ Contains biological material of animal origin

²⁾ Quantification Standard (positive control)

³⁾ No Template Control (negative control)

The AltoStar® HBV PCR Kit 1.5 contains enough reagents to perform 96 reactions.

3. Storage and handling

- The AltoStar® HBV 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.
- Do not exceed the following thaw-freeze-sequence for each master reagent tube: *Thaw 1 → Freeze 1 → Thaw 2*
- Do not exceed the following thaw-freeze-sequence for each Quantification Standard (QS) and No Template Control (NTC) tube: *Thaw 1 → Freeze 1 → Thaw 2 → Freeze 2 → Thaw 3 → Freeze 3 → Thaw 4*
- After thawing all components are stable for 5 hours at up to +30 °C.

4. Product description

The AltoStar® HBV PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the detection and quantification of hepatitis B virus (HBV) specific DNA.

Real-time PCR technology utilizes 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 HBV DNA specific amplification and detection system the AltoStar® HBV 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 HBV DNA are labeled with the fluorophore FAM™. The probe specific for the IC is labeled with a fluorophore (JOE™) detectable in the e.g. VIC™ channel.

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

4.1 Component description

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

The QS contain standardized concentrations of HBV specific DNA (see table 2). They were calibrated against the 4th WHO International Standard for HBV DNA for Nucleic Acid Amplification Techniques (NIBSC code: 10/266, genotype A2). The QS are used to verify the functionality of the HBV DNA specific amplification and detection system as well as to generate a standard curve, which allows the quantification of HBV specific DNA in a sample.

Table 2: Quantification Standards

Quantification Standard	Concentration [IU/μl]
QS1	1.00E+04
QS2	1.00E+03
QS3	1.00E+02
QS4	1.00E+01

The NTC contains no HBV specific DNA but does contain the IC template. The NTC is used as negative control for the HBV DNA specific real-time PCR and indicates possible contamination of Master A and Master B.

4.2 Real-time PCR instruments

The AltoStar® HBV PCR Kit 1.5 can be used with the following real-time PCR instrument:

- CFX96™ Deep Well Dx System (Bio-Rad)

NOTE



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® HBV 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® HBV PCR Kit 1.5:

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

6. Procedure

6.1 Sample preparation

Extracted DNA is the starting material for the AltoStar® HBV PCR Kit 1.5. The quality of the extracted DNA has a profound impact on the performance of the product.

The AltoStar® HBV 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 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.

For additional information and technical support regarding pre-treatment and sample preparation, contact Altona Diagnostics technical support (see chapter 9. Technical support).

6.2 Master mix setup

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

Set up the master mix according to the following pipetting scheme:

Table 3: Pipetting scheme (master mix setup)

Number of reactions (rxns)	1	12
Master A	20 µl	240 µl
Master B	25 µl	300 µl
Volume master mix	45 µl	540 µl

NOTE



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

6.3 Reaction setup

1. Pipette 45 µl of the master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
2. Add 45 µl of the sample (eluate from the nucleic acid extraction) or 45 µl of the controls (QS1–4 or NTC).

Table 4: Pipetting scheme (reaction setup)

Reaction setup	
Master mix	45 µl
Sample or control	45 µl
Total volume	90 µl

NOTE



Do not add the IC to the QS and the NTC reactions, respectively, provided with this product.

3. Make sure that for quantitative analysis QS1–4 and 1 NTC are used. For qualitative analysis make sure that at least QS4 and 1 NTC are used.
4. Thoroughly mix the samples and controls with the master mix by pipetting up and down.
5. Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
6. 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).

After completion of the PCR mix setup the PCR mix in a sealed PCR plate is stable at room temperature (max. +30 °C) for max. 30 minutes.

7. Programming the real-time PCR instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed programming instructions regarding the use of the AltoStar® HBV PCR Kit 1.5 on specific real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical support).

7.1 Settings

Define the following basic settings:

Table 5: Run settings

Settings	
Reaction volume	90 µl
Ramp rate	Default
Passive reference*	ROX™

* If applicable

7.2 Fluorescence detectors (dyes)

Define the following fluorescence detectors (dyes):

Table 6: Fluorescence detectors

Target	Detector name	Reporter	Quencher
HBV specific DNA	HBV	FAM™	(None)
IC	Internal Control	JOE™	(None)

7.3 Temperature profile and dye acquisition

Define the following temperature profile and dye acquisition:

Table 7: Temperature profile and dye acquisition

	Stage	Cycle repeats	Acquisition	Temperature [°C]	Time [min:s]
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			Yes	58	00:45
			-	72	00:15

8. Data analysis

For basic information regarding data analysis on specific real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the AltoStar® HBV PCR Kit 1.5 on different real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical support).

8.1 Interpretation of results

8.1.1 Qualitative analysis

Table 8: Qualitative analysis

Detection channel		Result interpretation
FAM™ (HBV)	JOE™ (IC)	
+	+/-*	HBV specific DNA detected.
-	+	No HBV specific DNA detected. Sample does not contain detectable amounts of HBV specific DNA.
-	-	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 HBV DNA load in the sample can lead to a reduced or absent IC signal.

8.1.2 Quantitative analysis

The AltoStar® HBV PCR Kit 1.5 includes 4 QS. In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with appropriate concentrations (see chapter 4.1 Component description). Using **standards** of known concentrations a standard curve for quantitative analysis can be generated.

$$C_t = m \cdot \log(N_0) + b$$

C_t = Threshold cycle
 m = Slope
 N_0 = Initial concentration
 b = Intercept

Derived from the standard curve positive samples of unknown concentrations can be quantified.

$$(C_t - b) / m$$

$$N_0 = 10$$

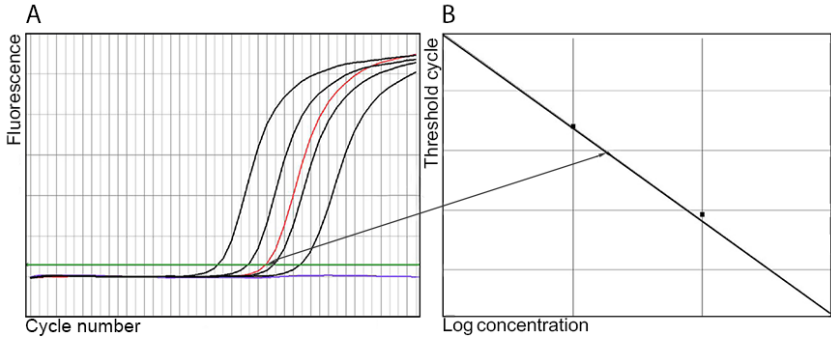


Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the amplification plot [A] and standard curve analysis [B]

NOTE



The concentration of the "sample" is displayed in IU/μl and refers to the concentration in the eluate.

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load (Sample) [IU/ml]} = \frac{\text{Volume (Eluate) [\mu l]} \cdot \text{Viral load (Eluate) [IU/\mu l]}}{\text{Sample input [ml]}}$$

9. Technical support

For customer support, contact altona Diagnostics technical support:

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

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

10. Trademarks and disclaimers
















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

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11. Symbols

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

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