altona DIAGNOSTICS

Instructions for use

AltoStar®
HIV RT-PCR Kit 1.5

05/2023 EN

AltoStar®

AltoStar®

HIV RT-PCR Kit 1.5

For research use only!

(RUO)

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AS0221503

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

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1. Application

The AltoStar® HIV RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the detection and quantification of human immunodeficiency virus type 1 (HIV-1) specific RNA.

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

2. Kit content

The AltoStar® HIV RT-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[2)]
Purple	Master B[1)]	8	300[3)]
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® HIV RT-PCR Kit 1.5 contains enough reagents to perform 96 reactions.

3. Storage and handling

- The AltoStar® HIV 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.
- 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® HIV RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the detection and quantification of human immunodeficiency virus type 1 (HIV-1) 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 HIV RNA specific amplification and detection systems the AltoStar® HIV 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 HIV RNA 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 HIV specific RNA and the IC in the corresponding detection channels of the real-time PCR instrument.

4.1 Component description

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 HIV specific RNA and the IC (AltoStar® Internal Control 1.5) in one reaction setup.

The QS contain standardized concentrations of HIV specific RNA (see table 2). They were calibrated against the 4th WHO International Standard for HIV-1 RNA (NIBSC code: 16/194). The QS are used to verify the functionality of the HIV RNA specific amplification and detection system as well as to generate a standard curve, which allows the quantification of HIV specific RNA 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 HIV specific RNA but does contain the IC template. The NTC is used as negative control for the HIV RNA specific real-time PCR and indicates possible contamination of Master A and Master B.

4.2 Real-time PCR instruments

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

- 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® HIV 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® HIV 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® HIV RT-PCR Kit 1.5. The quality of the extracted RNA has a profound impact on the performance of the product.

The AltoStar® HIV 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.

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 RT-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® HIV RT-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
HIV specific RNA	HIV	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]
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, refer to the instructions for use of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the AltoStar® HIV RT-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™ (HIV)	JOE™ (IC)	
+	+/-*	HIV specific RNA detected.
-	+	No HIV specific RNA detected. Sample does not contain detectable amounts of HIV 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 HIV RNA load in the sample can lead to a reduced or absent IC signal.

8.1.2 Quantitative analysis

The AltoStar® HIV RT-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.

$$N_0 = 10^{(C_t - b)/m}$$

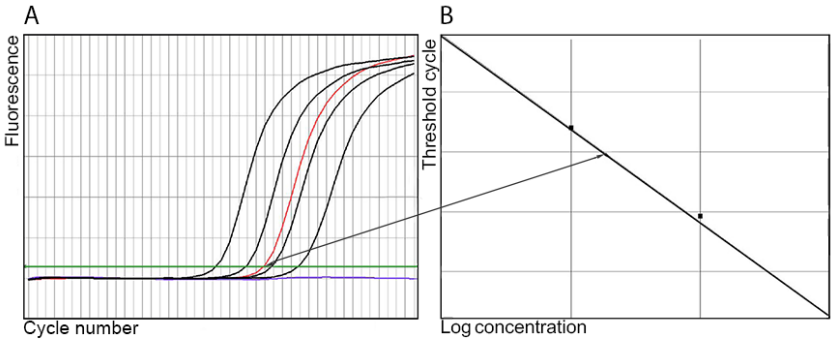

Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the amplification plot [**A**] and standard curve analysis [**B**]

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:

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

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); ABI Prism®, QuantStudio™ (Applied Biosystems); CFX96™ (Bio-Rad); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, JOE™, ROX™, VIC™ (Thermo Fisher Scientific).

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11. 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.

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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