altona DIAGNOSTICS

Instructions for use

AltoStar®
Pneumocystis jirovecii PCR Kit 1.5

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AltoStar®

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Pneumocystis jirovecii PCR Kit 1.5

For research use only!

(RUO)

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Content

1. Application

The AltoStar® Pneumocystis jirovecii PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection and quantification of *Pneumocystis jirovecii* specific DNA.

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

2. Kit content

The AltoStar® Pneumocystis jirovecii 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	60
Purple	Master B[1)]	8	180
Red	QS1[2)]	2	250
Red	QS2[2)]	2	250
Red	QS3[2)]	2	250
Red	QS4[2)]	2	250
White	NTC[3)]	2	250

[1)] Contains biological material of animal origin

[2)] Quantification Standard (positive control)

[3)] No Template Control (negative control)

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

3. Storage

- The AltoStar® Pneumocystis jirovecii 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® Pneumocystis jirovecii PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection and quantification of *Pneumocystis jirovecii* 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 *Pneumocystis jirovecii* DNA specific amplification and detection systems the AltoStar® Pneumocystis jirovecii 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 *Pneumocystis jirovecii* 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 *Pneumocystis jirovecii* 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, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and detection of *Pneumocystis jirovecii* specific DNA and the IC (AltoStar® Internal Control 1.5) in one reaction setup.

The Quantification Standards (QSs) contain standardized concentrations of *Pneumocystis jirovecii* specific DNA (see table 2). The material used for the QSs included in the AltoStar® Pneumocystis jirovecii PCR Kit 1.5 is traced to internally produced reference material. The QSs are used to verify the functionality of the *Pneumocystis jirovecii* DNA specific amplification and detection system as well as to generate a standard curve, which allows the quantification of *Pneumocystis jirovecii* specific DNA in a sample.

Table 2: Quantification Standards

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

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

4.2 Real-time PCR instruments

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

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

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® Pneumocystis jirovecii 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® Pneumocystis jirovecii 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® Pneumocystis jirovecii PCR Kit 1.5. The quality of the extracted DNA has a profound impact on the performance of the product.

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.

The AltoStar® Pneumocystis jirovecii 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.

1. 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.

2. 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	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

1. 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.
2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (QS1–4 or NTC).
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).

Table 4: Pipetting scheme (reaction setup)

Reaction setup	
Master mix	20 µl
Sample or control	10 µl
Total volume	**30 µl**

NOTE

Do not add the IC to the QS 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, refer to the instructions for use of the respective instrument.

For detailed programming instructions regarding the use of the AltoStar® Pneumocystis jirovecii 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	30 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
Pneumocystis jirovecii specific DNA	P. jirovecii	FAM™	(None)
Internal control	IC	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:sec]
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® Pneumocystis jirovecii 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™ (P. jirovecii)	JOE™ (IC)	
+	+/-*	*Pneumocystis jirovecii* specific DNA detected.
-	+	No *Pneumocystis jirovecii* specific DNA detected. Sample does not contain detectable amounts of *Pneumocystis jirovecii* 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 target DNA load in the sample can lead to reduced or absent IC signal.

8.1.2 Quantitative analysis

The AltoStar® Pneumocystis jirovecii PCR Kit 1.5 includes 4 QSs. In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with appropriate concentrations (see chapter 4. Product 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.

$$(C_t - b) / m$$

$$\mathbf{N_0 = 10}$$

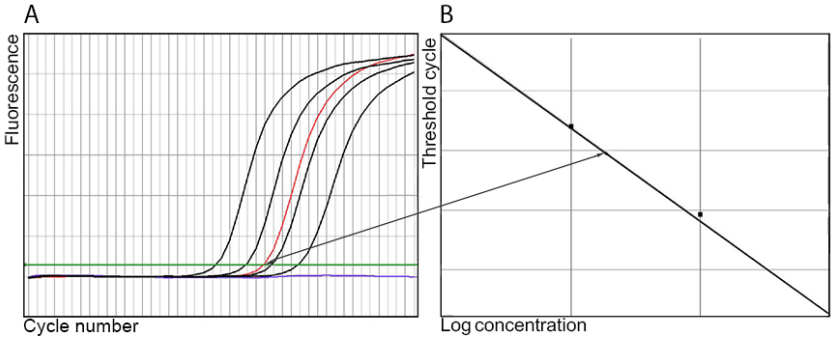

Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the amplification plot [**A**] and standard curve analysis [**B**]

NOTE

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

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

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

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); JOE™ (Life Technologies); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, ROX™ (Thermo Fisher Scientific).

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

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.

Symbol	Explanation
	Contains biological material of animal origin

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always a drop ahead.

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