

## **Instructions for Use**

# **RealStar<sup>®</sup> Pneumocystis jirovecii PCR Kit 1.0**

01/2022 EN



# RealStar®

## Pneumocystis jirovecii PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

LightCycler® 480 Instrument II (Roche)



551013



96



01 2022



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

## Content

<b>1.</b>	<b>Intended Use</b> .....	<b>6</b>
<b>2.</b>	<b>Kit Components</b> .....	<b>6</b>
<b>3.</b>	<b>Storage</b> .....	<b>6</b>
<b>4.</b>	<b>Material and Devices required but not provided</b> .....	<b>7</b>
<b>5.</b>	<b>Background Information</b> .....	<b>8</b>
<b>6.</b>	<b>Product Description</b> .....	<b>9</b>
6.1	Real-Time PCR Instruments .....	11
<b>7.</b>	<b>Warnings and Precautions</b> .....	<b>11</b>
<b>8.</b>	<b>Procedure</b> .....	<b>12</b>
8.1	Sample Preparation .....	12
8.2	Master Mix Setup .....	14
8.3	Reaction Setup.....	15
<b>9.</b>	<b>Programming the Real-Time PCR Instrument</b> .....	<b>16</b>
9.1	Settings .....	16
9.2	Fluorescence Detectors (Dyes).....	17
9.3	Temperature Profile and Dye Acquisition .....	17
<b>10.</b>	<b>Data Analysis</b> .....	<b>18</b>
10.1	Validity of Diagnostic Test Runs .....	18
10.1.1	Valid Diagnostic Test Run (qualitative).....	18
10.1.2	Invalid Diagnostic Test Run (qualitative) .....	18
10.1.3	Valid Diagnostic Test Run (quantitative).....	19
10.1.4	Invalid Diagnostic Test Run (quantitative).....	19

10.2	Interpretation of Results .....	20
10.2.1	Qualitative Analysis .....	20
10.2.2	Quantitative Analysis .....	20
<b>11.</b>	<b>Performance Evaluation .....</b>	<b>22</b>
11.1	Analytical Sensitivity .....	22
11.2	Analytical Specificity .....	23
11.3	Linear Range .....	23
11.4	Precision .....	25
<b>12.</b>	<b>Limitations .....</b>	<b>26</b>
<b>13.</b>	<b>Quality Control .....</b>	<b>27</b>
<b>14.</b>	<b>Technical Assistance .....</b>	<b>27</b>
<b>15.</b>	<b>Literature .....</b>	<b>27</b>
<b>16.</b>	<b>Trademarks and Disclaimers .....</b>	<b>28</b>
<b>17.</b>	<b>Explanation of Symbols .....</b>	<b>29</b>

## 1. Intended Use

The RealStar® *Pneumocystis jirovecii* PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of *Pneumocystis jirovecii* specific DNA.

## 2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	QS1-4*	4	250
White	Water (PCR grade)	1	500

\* The RealStar® *Pneumocystis jirovecii* PCR Kit 1.0 contains Quantification Standards (QS) at four different concentrations (see Chapter 6. Product Description)

## 3. Storage

- The RealStar® *Pneumocystis jirovecii* PCR Kit 1.0 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 between -25°C and -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. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

#### 4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.2 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

#### NOTE



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



It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

## 5. Background Information

*Pneumocystis jirovecii* is the causative agent of Pneumocystis pneumonia (PCP), one of the most frequent and severe opportunistic infections in immunocompromised individuals. The symptoms of PCP include dyspnea, nonproductive cough, and fever. In untreated PCP increasing pulmonary involvement leads to death. *Pneumocystis* is an obligate extracellular pathogen that exists in trophic and cystic forms. Initially classified as a protozoan, it was reclassified as a fungus based on greater DNA sequence homology with fungal organisms. *Pneumocystis* organisms represent a large group of species with worldwide distribution and a strong specificity for a given mammalian host species. Historically, all forms of *Pneumocystis* were referred to as *Pneumocystis carinii*. In recognition of the specificity of different organisms, the human form was renamed *P. jirovecii*. *P. carinii* is now reserved for the rat form of *Pneumocystis*.

Evidence from different studies suggests that *Pneumocystis* is transmitted by an airborne route and that transmission requires a short period of exposure and low numbers of organisms. Most human beings become seropositive to *P. jirovecii* organisms or antigens by the ages of 2 to 4 years. *P. jirovecii* can be detected in populations without underlying immunosuppression. Colonization, carriage, asymptomatic infection and subclinical infection have all been used to describe the presence of *Pneumocystis* organisms or DNA in the absence of PCP. The effects of colonization on the host and in mild respiratory infections, chronic lung disease, and progression to PCP have yet to be determined. Also, it is still under debate, if reactivation of latent infection is the primary cause of PCP or if person to person transmission is a key component of the disease process. According to the reactivation theory, *Pneumocystis* is commonly encountered in the environment during childhood. The organism does not cause clinical disease but it harbors within the host and can subsequently reactivate to cause PCP, if the host's immune function declines. On the other hand, it has recently been recognized that *de novo* exposure either from the environment or from individuals with PCP or colonized with *Pneumocystis* may result in transmission.



Because *Pneumocystis* cannot be cultured, diagnosis relies traditionally in visualization of the organism in respiratory samples, including induced sputum, BAL fluid, or lung tissue, or in the specific detection of *Pneumocystis* DNA, by using more sensitive molecular diagnostic assays.

Selection of an initial anti *Pneumocystis* regimen depends on the severity of the patient illness, other comorbid conditions, and the patient ability to tolerate a specific agent.

## 6. Product Description

The RealStar® *Pneumocystis jirovecii* PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of *Pneumocystis jirovecii* specific DNA.

The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

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 labelled with fluorescent reporter and quencher dyes.

Probes specific for *P. jirovecii* DNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of *P. jirovecii* specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Four Quantification Standards (QS1 - QS4)
- PCR grade water

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 *P. jirovecii* specific DNA and Internal Control in one reaction setup.

The Quantification Standards contain standardised concentrations of *P. jirovecii* specific DNA. The Quantification Standards can be used individually as positive controls, or together to generate a **standard curve**, which can be used to determine the concentration of *P. jirovecii* specific DNA in the sample.

The Quantification Standards have the following concentrations:

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

## 6.1 Real-Time PCR Instruments

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

## 7. Warnings and Precautions

*Read the Instructions for Use carefully before using the product.*

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.

- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

## 8. Procedure

### 8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® Pneumocystis jirovecii PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIASymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with Realstar® Pneumocystis jirovecii PCR Kit 1.0 has to be validated by the user.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

### CAUTION



**If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.**



**The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.**

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

## 8.2 Master Mix Setup

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

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

- ▶ If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, 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
Internal Control	1 µl	12 µl
<b>Volume Master Mix</b>	<b>21 µl</b>	<b>252 µl</b>

- ▶ If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.
- ▶ No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.
- ▶ If the IC was added during the sample preparation procedure, 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
<b>Volume Master Mix</b>	<b>20 µl</b>	<b>240 µl</b>

### CAUTION



If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.



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

## 8.3 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 (Quantification Standard, Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
<b>Total Volume</b>	<b>30 µl</b>

- ▶ Make sure that at least one Positive (QS) and one Negative Control is used per run.
- ▶ For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- ▶ 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 1000 x g (~ 3000 rpm).

## 9. 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 RealStar® Pneumocystis jirovecii PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

### 9.1 Settings

- ▶ Define the following settings:

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



## 9.2 Fluorescence Detectors (Dyes)

- Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
<i>P. jirovecii</i> specific DNA	<i>P. jirovecii</i>	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

## 9.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Analysis Mode	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

## 10. 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 RealStar® Pneumocystis jirovecii PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

### 10.1 Validity of Diagnostic Test Runs

#### 10.1.1 Valid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel	
	FAM™	JOE™
Positive Control (QS)	+	+/-*
Negative Control	-	+

\* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

#### 10.1.2 Invalid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

### 10.1.3 Valid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **valid**, if all control conditions for a **valid qualitative** diagnostic test run are met [see chapter 10.1.1 Valid Diagnostic Test Run (qualitative)]. The **quantification** results are **valid** if the generated **standard curve** reaches the following control parameter value:

Control Parameter	Valid Value
R square ( $R^2$ )	$\geq 0.98$

#### NOTE



Not all real-time PCR instruments display the R square ( $R^2$ ) value. For detailed information, please refer to the user manual of the respective instrument.

### 10.1.4 Invalid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid quantitative** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

## 10.2 Interpretation of Results

### 10.2.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	<i>P. jirovecii</i> specific DNA detected.
-	+	No <i>P. jirovecii</i> specific DNA detected. Sample does not contain detectable amounts of <i>P. jirovecii</i> specific DNA.
-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

\* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high *P. jirovecii* DNA load in the sample can lead to a reduced or absent Internal Control signal.

### 10.2.2 Quantitative Analysis

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 includes four Quantification Standards (QS). In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with appropriate concentrations (see chapter 6. 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

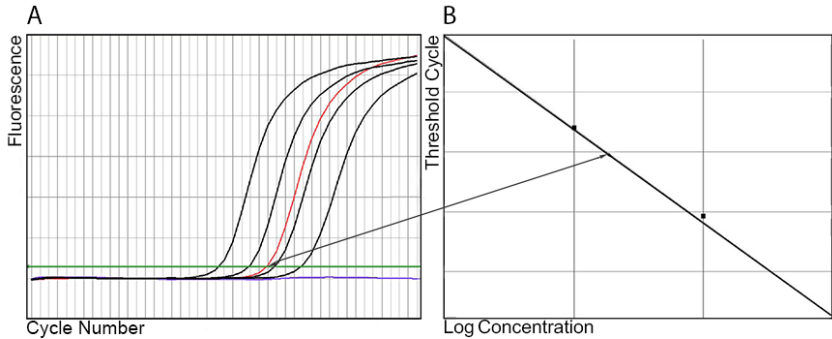
$m$  = Slope

$N_0$  = Initial Concentration

$b$  = Intercept

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

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



**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 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:

$$\text{Fungal load (Sample) [copies/ml]} = \frac{\text{Volume (Eluate) } [\mu\text{l}] \cdot \text{Fungal load (Eluate) [copies}/\mu\text{l}]}{\text{Sample Input [ml]}}$$

## 11. Performance Evaluation

### 11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 is defined as the concentration (copies/μl of the eluate) of *P. jirovecii* specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified *P. jirovecii* DNA.

**Table 1:** PCR results used for the calculation of the analytical sensitivity with respect to the detection of *P. jirovecii* specific DNA

Input Conc. [copies/μl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.622	16	16	100
10.000	16	16	100
3.162	24	24	100
1.000	24	24	100
0.5	24	24	100
0.316	24	24	100
0.100	24	13	54
0.032	24	7	29
0.010	24	1	4
0.003	24	0	0

The analytical sensitivity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was determined by Probit analysis:

- For the detection of *P. jirovecii*. specific DNA, the analytical sensitivity is 0.29 copies/μl [95% confidence interval (CI): 0.19 - 0.55 copies/μl].

## 11.2 Analytical Specificity

The analytical specificity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant *P. jirovecii* genotypes will be detected.

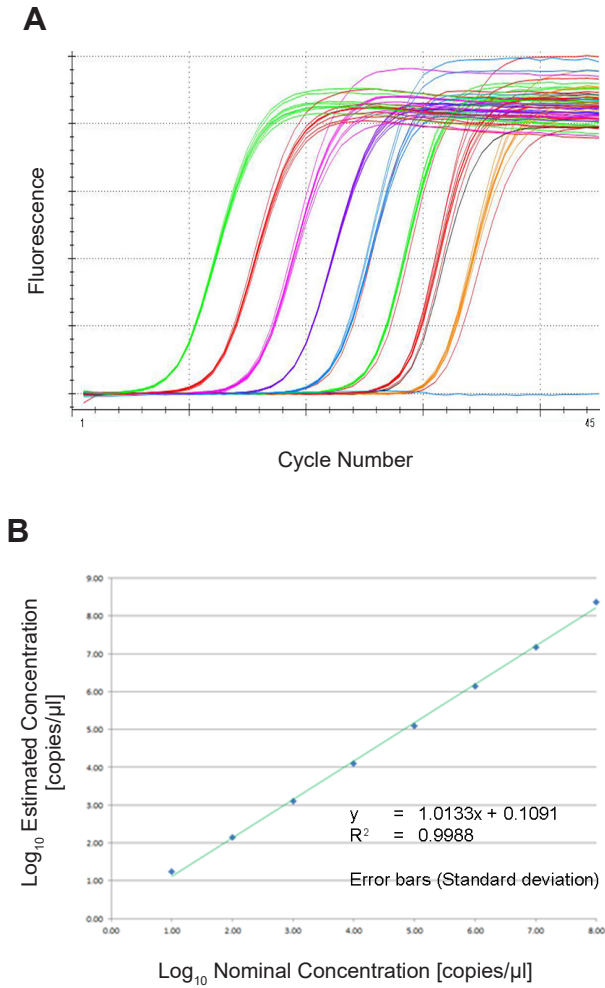
The analytical specificity of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was evaluated by testing a panel of genomic DNA/RNA extracted from pathogens causing similar symptoms as Pneumocystis jirovecii.

The RealStar® Pneumocystis jirovecii PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis E virus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6A
- Human herpesvirus 6B
- Human immunodeficiency virus 1
- Human parvovirus B19
- Influenza A virus H1N1<sub>nv</sub>
- Influenza A virus H3N2
- Influenza B virus
- Varicella-zoster virus
- *Chlamydomphila pneumoniae*
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Mycobacterium tuberculosis*
- *Mycoplasma pneumoniae*
- *Neisseria meningitidis*
- *Pneumocystis carinii*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

## 11.3 Linear Range

The linear range of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was evaluated by analyzing a logarithmic dilution series of Pneumocystis jirovecii specific DNA using concentrations ranging from 10<sup>8</sup> copies/μl to 10<sup>1</sup> copies/μl. At least eight replicates per dilution were analysed.



**Figure 2:** Amplification curves **[A]** and linear regression **[B]** of an analysed dilution series of *P. jirovecii* specific DNA

The linear range of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was determined to be 10<sup>1</sup> copies/μl to 10<sup>8</sup> copies/μl.



## 11.4 Precision

Precision of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analyses.

Variability data are expressed in terms of standard deviation and coefficient of variation. The data are based on quantification analysis of defined concentrations of *P. jirovecii* specific DNA and on threshold cycle ( $C_t$ ) value in terms of the Internal Control. At least six replicates per sample were analysed for intra-assay, inter-assay and inter-lot variability.

**Table 2:** Precision data for the detection of *P. jirovecii* specific DNA

<i>P. jirovecii</i>	Average Conc. [copies/ $\mu$ l]	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	3.72	0.01	0.26
Inter-Assay Variability	3.76	0.05	1.35
Inter-Lot Variability	3.74	0.02	0.62
Total Variability	3.76	0.04	1.06

**Table 3:** Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle ( $C_t$ )	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	26.20	0.05	0.21
Inter-Assay Variability	26.18	0.07	0.29
Inter-Lot Variability	26.16	0.08	0.32
Total Variability	26.16	0.08	0.31

## 12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause underquantification, false negative or invalid results.
- Potential mutations within the target regions of the *P. jirovecii* genome covered by the primers and/or probes used in the kit may result in underquantification and/or failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® Pneumocystis jirovecii PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

### 13. Quality Control

In accordance with the Altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® Pneumocystis jirovecii PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

### 14. Technical Assistance

For technical advice, please contact our Technical Support:

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

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

### 15. Literature

Versalovic, James, Carroll, Karen C., Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

## 16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); SmartCycler® (Cepheid); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, MinElute®, QIASymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.
















The RealStar® Pneumocystis jirovecii PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not FDA cleared or approved.

Not available in all countries.

© 2022 altona Diagnostics GmbH; all rights reserved.

## 17. Explanation of Symbols

Symbol	Explanation
	<i>In vitro</i> diagnostic medical device
	Batch code
	Cap color
	Product number
	Content
	Number
	Material number
	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Version

**Notes:**



**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](mailto:info@altona-diagnostics.com)

[www.altona-diagnostics.com](http://www.altona-diagnostics.com)

