Instructions for Use

LyteStar™ Rickettsial Diseases PCR Kit 1.0

LyteStar™ Rickettsial Diseases PCR Kit 1.0

For detection of Pan-*Rickettsia* and Pan-*Orientia* from human specimens

For use with

Rotor-Gene Q5/6 plex Platform (Qiagen)
CFX96™ (BioRad)
CFX Opus 96 (BioRad)
Magnetic Induction Cycler (Mic; Bio Molecular Systems)
*ab*CyclerQ (AITbiotech)

Product No.: 887103

96 reactions

Please refer to Storage and Shelf Life in this IFU

Ver. 01 / June 2025

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Content

1. Intended Use

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 is intended for the specific detection and differentiation of pan-*Rickettsia* and pan-*Orientia* DNA in human whole blood, serum, and eschar tissue (biopsy or swab), for the diagnosis of spotted fever, typhus fever and scrub typhus infections. The LyteStar™ Rickettsial Diseases PCR Kit 1.0 is a dual-target assay comprising screening assays targeting the pan-*Orientia* 16S rRNA (*rrs*) gene and the pan-*Rickettsia* citrate synthase (*gltA*) gene.

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 is for professional use only.

2. Kit Components

Catalog no.	887103
Master A	4 x 300 µl
Master B	2 x 360 µl
Internal Control (IC)	800 µl
Positive Control (PC)	200 µl
PCR grade water	500 µl

3. Storage and Shelf Life

- The LyteStar™ Rickettsial Diseases PCR Kit 1.0 has a shelf life of 12 months from the manufacturing date.
- Store all reagents at -15°C to -25°C upon arrival.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay. Master B should be frozen in aliquots, if they are to be used intermittently.
- Mix Master A thoroughly by vortex mixing, and centrifuge briefly before use.
- Protect Master B from light.
- All frozen reagents should be completely thawed to room temperature before use. Immediately return unused portions to the freezer for storage.

4. Quality Control

In compliance with AstronDX Technologies' EN ISO 13485 certified Quality Management System, each lot of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 is tested against pre-determined specifications to ensure consistent product quality.

5. Product Use Limitations and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR procedures.
- Specimens should always be treated as if 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 specimen 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) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities.
- Workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering different areas.
- 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 use components of the kit that have passed their expiration date.

- Discard sample and assay waste according to your local safety regulations.
- Wash hands thoroughly after handling specimens and test reagents.
- Do not use kits from different lots together.
- Do not use an expired kit.
- In case of damage to the packaging and leaking vials, do not use the kit (possible contamination or deterioration that can cause false interpretation).

6. Product Warranty

AstronDX Technologies guarantees the performance of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 for applications as described in the manual. The user must determine the suitability of the product for the particular intended use. Should the product fail to perform satisfactorily in the described applications, please contact AstronDX Technologies Technical Support (**16. Technical Support**) for trouble-shooting.

AstronDX Technologies reserves the right to change, alter, or modify any product to enhance its performance and design.

7. Product Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles/face masks. For more information, please consult the appropriate material safety data sheets (MSDSs).

8. Introduction

Rickettsial diseases are vector-borne diseases caused by agents of the order Rickettsiales, which include the genera *Rickettsia* and *Orientia* from the family Rickettsiaceae [1]. Both *Rickettsia* and *Orientia* represent two closely related evolutionary lineages that once were considered as belonging to the same genus [2]. They are gram-negative, obligately intracellular bacteria that are usually transmitted to humans by arthropods, such as ticks, fleas, lice, or mites [3].

The genus *Rickettsia* is classified into two major antigenic groups, namely the spotted fever group (SFG) and the typhus group (TG) [2]. The SFG encompasses at least 32 tick-borne species, of which nearly 15 species, including the two most pathogenic and well-studied *Rickettsia rickettsii* (the agent of Rocky Mountain

spotted fever) and *Rickettsia conorii* (the agent of Mediterranean spotted fever), have been implicated in human disease [3,4]. On the other hand, the TG includes two human pathogenic species, which are the louse-borne *Rickettsia prowazekii* (the agent of epidemic typhus) and the flea-borne *Rickettsia typhi* (the agent of endemic typhus) [2].

The genus *Orientia* comprises the scrub typhus group (STG) that causes the human mite-borne disease scrub typhus [5]. STG initially comprised of a single species, *Orientia tsutsugamushi*, which is prevalent in Asia [3]. Two additional species have since been described, *Orientia chuto* isolated from a patient in Dubai [6] and *Orientia chiloensis* discovered in southern Chile [7].

Both *Rickettsia* and *Orientia* infections present clinically with headache, fever, and rash, although additional clinical symptoms are species-specific, such as the presence of a necrotic lesion or eschar at the site of infection, which is commonly seen in scrub typhus and some SFG rickettsial infections such as African tick bite fever [4,5]. Despite rickettsial infections being among the leading aetiologies of acute febrile illness in Southeast Asia, their impact remains under appreciated. This is in part due to their undifferentiated clinical manifestations, which are often indistinguishable from other acute febrile illnesses during early stage of illness [3,8]. This emphasizes the need for early accurate diagnosis of rickettsial infections to avoid a missed diagnosis that may lead to more severe outcomes and death.

Apart from a potentially missed diagnosis, the increasing incidence of rickettsial diseases worldwide necessitates rapid and accurate diagnostic tools for a broad range of rickettsial agents causing spotted fever, typhus fever and scrub typhus, which is important for the rapid administration of appropriate antibiotics. As such, the LyteStar™ Rickettsial Diseases PCR Kit 1.0 was developed to rapidly detect and differentiate pan-*Rickettsia* (SFG and TG) and pan-*Orientia* (STG) in a single assay.

[1] Jiang, J., Farris, C. M., Yeh, K. B., & Richards, A. L. (2021). International Rickettsia disease surveillance: An example of cooperative research to increase laboratory capability and capacity for risk assessment of Rickettsial outbreaks worldwide. *Frontiers in Medicine, 8,* 622015. https://doi.org/10.3389/fmed.2021.622015

[2] Liu, D. (2015). Rickettsia. In Y.-W. Tang, M. Sussman, D. Liu, I. Poxton, & J. Schwartzman (Eds.), *Molecular Medical Microbiology* (2nd ed., pp. 2043-2056). Academic Press.

[3] Paris, D. H., Richards, A. L., & Day, N. P. J. (2015). Orientia. In Y.-W. Tang, M. Sussman, D. Liu, I. Poxton, & J. Schwartzman (Eds.), *Molecular Medical Microbiology* (2nd ed., pp. 2057-2096). Academic Press.

[4] Premaratna, R. (2022). Rickettsial illnesses, a leading cause of acute febrile illness. *Clinical Medicine (London, England),* 22(1), 2–5. https://doi.org/10.7861/clinmed.2021-0790

[5] Gillespie, J. J., & Salje, J. (2023). Orientia and Rickettsia: Different flowers from the same garden. *Current Opinion in Microbiology,* 74, 102318. Advance online publication. https://doi.org/10.1016/j.mib.2023.102318

[6] Izzard, L., Fuller, A., Blacksell, S. D., Paris, D. H., Richards, A. L., Aukkanit, N., Nguyen, C., Jiang, J., Fenwick, S., Day, N. P., Graves, S., & Stenos, J. (2010). Isolation of a novel Orientia species (O. chuto sp. nov.) from a patient infected in Dubai. *Journal of Clinical Microbiology,* 48(12), 4404–4409. https://doi.org/10.1128/JCM.01526-10

[7] Abarca, K., Martínez-Valdebenito, C., Angulo, J., Jiang, J., Farris, C. M., Richards, A. L., Acosta-Jamett, G., & Weitzel, T. (2020). Molecular description of a novel Orientia species causing scrub typhus in Chile. *Emerging Infectious Diseases,* 26(9), 2148–2156. https://doi.org/10.3201/eid2609.200918

[8] Blanton L. S. (2019). The Rickettsioses: A practical update. I*nfectious Disease Clinics of North America, 33*(1), 213–229. https://doi.org/10.1016/j.idc.2018.10.010

9. Product Description

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 is an *in vitro* diagnostic test system, based on real-time PCR technology, for the qualitative detection and differentiation of pan-*Rickettsia* and pan-*Orientia* DNA. The kit consists of a single tube assay targeting two genes, the 16S rRNA (*rrs*) gene for the detection of scrub typhus group (STG) *Orientiae,* and the citrate synthase (*gltA*) gene for the detection of spotted fever (SFG) and typhus group (TG) *Rickettsiae*. 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. The Internal Control template used in the LyteStar™ Rickettsial Diseases PCR Kit 1.0 is an artificial sequence with no homology to any known genomes.

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 utilizes real-time polymerase chain reaction technology 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. The probes used for specific amplification of *rrs* gene of pan-*Orientia* DNA and *gltA* gene of pan-*Rickettsia* DNA are labelled with the fluorophore FAM and Cy5™, respectively. The *rrs* gene probe detects all *Orientia* species including *Orientia tsutsugamushi, Orientia chuto* and *Orientia chiloensis*, while the *gltA* gene probe detects all *Rickettsia* species of STG and TG. The probe specific to the target of the Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dye enable the parallel detection of pan-*Orientia*, pan-*Rickettsia* and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides included in the two assays were designed, modified, and based on sequences/target regions published in the articles below:

Target	**Publication**
Pan-*Rickettsia*	Stenos *et al*., (2005)
Pan-*Orientia*	Jiang *et al*., (2022)
Internal Control	Deer *et al*., (2010)

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

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

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- The template of the Internal Control (IC)
- The template of the Positive Control (PC)
- PCR grade water (for setting up of “No Template Control”, NTC)

Master A and Master B reagents contain all components (buffer, enzymes, primers and probes) to allow PCR mediated amplification and target detection of *rrs* gene of pan-*Orientia* specific DNA, *gltA* gene of pan-*Rickettsia* specific DNA, and Internal Control in one reaction setup.

The Positive Control (PC) contains synthesized target genes of pan-*Orientia* and pan-*Rickettsia*.

The Internal Control used in the LyteStar™ Rickettsial Diseases PCR Kit 1.0 is DNA of an artificial sequence with no homology to any known genomes.

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Rotor-Gene Q 5/6 plex Platform (Qiagen)
- CFX96™ (BioRad)
- CFX Opus 96 (BioRad)
- Magnetic Induction Cycler (Mic: Bio Molecular Systems)
- *ab*CyclerQ (AITbiotech)

10. Material and Devices required but Not Provided

- Appropriate real-time PCR instrument
- Appropriate nucleic acid extraction system or kit
- 1.5 ml microcentrifuge tubes (with safe-lock or screw cap)
- Microcentrifuge (with speed ≥ 13,000 rpm)
- Pipettes, adjustable (range: 10 µl, 100 µl, 200 µl, 1000 µl)
- Pipette tips (with aerosol barriers)
- Disposable gloves (powder-free)
- Heating block for lysis of specimens during extraction
- Vortex mixer
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material

11. Specimen Storage

- Suitable specimens include whole blood, serum, plasma and eschar tissues (biopsy or swab).
- Follow specimen transport and storage conditions outlined in the following guidelines:
  - ➢ World Health Organization (2000). Guidelines for the collection of clinical specimens during field investigation of outbreaks.

https://apps.Who.int/iris/handle/10665/66346

- Centers for Disease Control and Prevention (2022). Instructions for Submitting Diagnostic Specimens for Testing by the Rickettsial Reference Diagnostic Laboratory. https://www.cdc.gov/ncezid/dvbd/specimensub/rickettsial-shipping.html

- Centers for Disease Control and Prevention (2022). Sample Specifications, Storage, and Packaging Guidelines for Rickettsial Testing. https://www.cdc.gov/ncezid/dvbd/pdf/FS_SpecimenSubmissionGuidelines-508.pdf

12. Instructions for Use

12.1. Sample Preparation

Extracted DNA is the starting material for the LyteStar™ Rickettsial Diseases PCR Kit 1.0. The quality of the extracted DNA has a profound impact on the performance of the whole test system. It has to be ensured that the nucleic acid extraction system used is compatible with real-time PCR technology.

The following nucleic acid extraction kits / systems are suitable for use with the LyteStar™ Rickettsial Diseases PCR Kit 1.0:

- SpinStar™ Total DNA Kit 2.0 (AstronDX Technologies)
- MagCore® Plus II Automated Nucleic Acid Extractor (RBC Bioscience)
- QIAamp® DNA Mini Kit (Qiagen)
- QIAsymphony® (Qiagen)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with LyteStar™ Rickettsial Diseases 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, an additional centrifugation step for 10 min at approximately 17000 × g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid is highly recommended.

NOTE

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

12.2. Master Mix Setup

1. All reagents and samples should be thawed completely, mixed (by gentle vortex mixing) and centrifuged briefly before use. Prepare a marginal excess (additional 0.5 reaction) of the required Master Mix volume.

2. The LyteStar™ Rickettsial Diseases PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as (i) a PCR inhibition control or as (ii) a control of the sample preparation procedure (nucleic acid extraction) and PCR inhibition control.

   (i) If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	12.5 µl	150 µl
Master B	7.5 µl	90 µl
IC	0.5 µl	6 µl
Volume Master Mix	**20.5 µl**	**246 µl**

   (ii) If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, the IC has to be added 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 depends always and only 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 must be added into the specimen/lysis buffer mixture.

If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	12.5 µl	150 µl
Master B	7.5 µl	90 µl
Volume Master Mix	**20 µl**	**240 µl**

NOTE

⚠ ***Never add the Internal Control directly to the specimen.***

12.3. Reaction Setup

1. Pipette 20 µl Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

2. Add 5 µl of the sample (eluate from the nucleic acid extraction) or 5 µl of the controls (Positive Control; or water as No Template Control, NTC).

3. Make sure at least one Positive Control and one NTC are used per run.

4. Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

5. Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate caps.

6. Centrifuge the 96-well reaction plate at 1,000 x g (~3,000 rpm) for 30s.

Reaction Setup	
Master Mix	20 µl
Sample or Control	5 µl
Total Volume	**25 µl**

13. Programming the Real-Time PCR Instrument

13.1 Settings

• Define the following settings:

Settings	
Reaction Volume	25 µl
Ramp Rate	Default

13.2 Fluorescent Detectors (Dyes)

• Define the following fluorescent detectors:

	Detector Name	Reporter	Quencher
Pan-*Orientia (rss* gene) specific DNA	Pan-*Orientia*	FAM	IBFQ
Pan-*Rickettsia (gltA* gene) specific DNA	Pan-*Rickettsia*	Cy5	IBRQ
Internal Control	IC	HEX	IBFQ

13.3 Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Initial Activation	Hold	1	-	95 °C	2:00 min
Denaturation	Cycling	45	-	95 °C	15 sec
Annealing			√	60 °C	60 sec

√ Signal acquisition: activate FAM, Cy5, and HEX channels in all runs.

14. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument. For detailed instructions regarding data analysis of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (**16.Technical Support**).

14.1. Validity of Diagnostic Test Runs

14.1.1 Valid Diagnostic Test Runs (Qualitative)

For a **valid** diagnostic test run (qualitative), the following control conditions must be met:

Control ID	FAM / Cy5 Detection Channel	HEX Detection Channel
Positive Control	POSITIVE	POSITIVE
Negative Control	NEGATIVE	POSITIVE

14.1.2 Target CT values of PC and IC

	Positive Control *(Pan-Orientia)*	Positive Control *(Pan-Rickettsia)*	Internal Control
Target CT value	< 35 cycles	< 35 cycles	≤ 40 cycles*

*Required for unknown samples that do not amplify in FAM and Cy5 channels

Note: The above CT target values are exclusively given for **monitoring the integrity of the product and validated assay conditions** and should be achieved **ONLY for the provided Positive Control (PC) and Internal Control (IC)** when used as per the instructions given under section 12.3. Reaction set up. **The target CT values for PC MUST NOT be misinterpreted as the diagnostic cut-off values for clinical samples.**

14.1.3 Invalid Diagnostic Test Runs (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.

14.2 Interpretation of Results

FAM *Pan-Orientia*	**Cy5** *Pan-Rickettsia*	**HEX** *Internal Control*	**Result Interpretation**
+	+	+*	Pan-*Orientia* and pan-*Rickettsia* specific DNA detected. *Positive for pan-Orientia and pan-Rickettsia*
+	-	+*	Pan-*Orientia* specific DNA detected. *Positive for pan-Orientia*
-	+	+*	Pan-*Rickettsia* specific DNA detected. *Positive for pan-Rickettsia*
-	-	+	Both pan-*Orientia* and pan-*Rickettsia* specific DNA not detected. The sample does not contain detectable amounts of pan-*Orientia* and pan-*Rickettsia* specific DNA.
-	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For pan-*Orientia rrs* gene (FAM channel) and pan-*Rickettsia gltA* gene (Cy5 channel) "+" refers to amplification curve detected at CT ≤ 45 cycles. "-" refers to no amplification / no CT obtained.

* Detection of the Internal Control in the HEX channel is not required for positive results in the FAM/Cy5 detection channels. A high pan-*Orientia* and/or pan-*Rickettsia* load in the sample can lead to reduced or absent Internal Control signals.

14.2.1 Threshold Settings for Cycler Software

Cycler	Threshold		
	FAM Pan-*Orientia* Channel	FAM Pan-*Rickettsia* Channel	HEX IC Channel
Rotor-Gene	0.05 norm. fluoro	0.05 norm. fluoro	0.05 norm. fluoro
CFX96™	100 RFU	100 RFU	100 RFU
CFX Opus 96	100 RFU	100 RFU	100 RFU
Mic qPCR	Auto	Auto	Auto
*ab*CyclerQ	Auto	Auto	Auto

14.2.2 CT Cut-Off Values for Clinical Samples

	FAM Pan-*Orientia* Channel	Cy5 Pan-*Rickettsia* Channel
CT Cut-Off Value	< 45 cycles	< 45 cycles

15. Performance Evaluation

The analytical performance evaluation of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 was done using quantified pan-*Orientia* and pan-*Rickettsia* specific DNA.

15.1 Analytical Sensitivity

The analytical sensitivity (limit of detection: LoD) of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 is defined as the concentration of pan-*Orientia* and pan-*Rickettsia* DNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined by analyzing samples with known pan-*Orientia* and pan-*Rickettsia* concentration.

A dilution series of the AmpliRun® Orientia tsutsugamushi DNA control and AmpliRun® Rickettsia conorri DNA control was prepared by using 1× TE buffer as diluent. Dilutions of pan-*Orientia* and pan-*Rickettsia* DNA were tested with the LyteStar™ Rickettsial Diseases PCR Kit 1.0. Results were analyzed by Probit analysis (Table 1 and Table 2).

The analytical sensitivity of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 in combination with the Rotor-Gene Q 5-plex platform was determined at 2.23 copies/µl for pan-*Orientia rrs* gene target and 5.56 copies/µl for pan-*Rickettsia gltA* gene target (p≤ 0.05).

Table 1. PCR results used for the calculation of the analytical sensitivity of pan-*Orientia rrs* gene target for the LyteStar™ Rickettsial Diseases PCR Kit 1.0 in combination with the Rotor-Gene Q 5-plex platform (Qiagen).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
10.0	12	12	100
6.3	12	12	100
2.0	12	11	91.7
0.63	12	1	8.3
0.20	12	0	0
0.063	12	0	0
0.020	12	0	0
0.0063	12	0	0

Table 2. PCR results used for the calculation of the analytical sensitivity of pan-*Rickettsia gltA* gene target for the LyteStar™ Rickettsial Diseases PCR Kit 1.0 in combination with the Rotor-Gene Q 5-plex platform (Qiagen).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
10.0	12	12	100
6.3	12	12	100
2.0	12	6	50
0.63	12	3	25
0.20	12	0	0
0.063	12	0	0
0.020	12	0	0
0.0063	12	0	0

15.2 Analytical Specificity

The analytical specificity of the LyteStar™ Rickettsial Diseases 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 the applied primer/probes in LyteStar™ Rickettsial Diseases PCR Kit 1.0 specifically detect pan-*Orientia* and pan-*Rickettsia*.

The specificity of the LyteStar™ Rickettsial Diseases PCR Kit 1.0 was evaluated by testing genomic RNA/DNA extracted from other pathogens likely to be present in the same sample material as pan-*Orientia* and pan-*Rickettsia*, or that cause similar symptoms to these bacteria (Table 3).

Table 3. Microorganisms tested to demonstrate the analytical specificity of the LyteStar™ Rickettsial Diseases PCR Kit 1.0.

LyteStar™ Rickettsial Diseases RT-PCR Kit 1.0			
Organisms	***Pan-Orientia (FAM channel)***	***Pan-Rickettsia (Cy5 channel)***	***Internal Control (HEX channel)***
Borrelia burgdorferi	negative	negative	valid
Leptospira interrogans	negative	negative	valid
Legionella pneumophila	negative	negative	valid
Streptococcus pneumoniae	negative	negative	valid
Haemophilus influenzae	negative	negative	valid
Parvovirus B19 (PAB19)	negative	negative	valid
Epstein-Barr virus (EBV)	negative	negative	valid
Human herpesvirus 5 HCMV strain AD-169	negative	negative	valid
Yellow Fever virus (YFV)	negative	negative	valid
West Nile virus (WNV)	negative	negative	valid
Japanese Encephalitis virus (JEV)	negative	negative	valid
Zika virus (Asian lineage)	negative	negative	valid
Chikungunya virus (CHIKV)	negative	negative	valid
Dengue 1 virus	negative	negative	valid
Dengue 2 virus	negative	negative	valid
Dengue 3 virus	negative	negative	valid
Dengue 4 virus	negative	negative	valid
Orientia tsutsugamushi	positive	negative	valid
Rickettsia conorii	negative	positive	valid
Rickettsia monacensis strain IrR/Munich	negative	positive	valid

The LyteStar™ Rickettsial Diseases PCR Kit 1.0 did not cross-react with any pathogen or genotype/subtype other than its own target.

16. Technical Support

For customer support, please contact our Technical Support:

e-mail: **techsupport@astrondx.com**
phone: **+603 7931 6760**

17. Appendix

Explanation of Symbols

Symbol	Explanation
	Product Number
	Batch Code
	Manufacturer
	Date of Manufacture
	Contains sufficient for "n" tests/rxns
	Temperature limitation
	Version
	Use-By Date

18. Ordering Information

Products	Packing (reactions)	Order No.
LyteStar™ Rickettsial Diseases PCR Kit 1.0	96	887103
SpinStar™ Total DNA Kit 2.0	100	821803
MagCore® Genomic DNA Tissue Kit, CART CODE 401	96	MGT-02

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