Instructions for Use

LyteStar™
TB-MDR PCR Kit 1.0

LyteStar™
TB-MDR PCR Kit 1.0

For detection and differentiation of rifampicin and isoniazid-resistant tuberculosis DNA, in *Mycobacterium tuberculosis* positive human specimens

For use with

Rotor-Gene Q5/6 Plex Platform (Qiagen)
CFX96™ (BioRad)
CFX Opus 96 (BioRad)
Magnetic Induction Cycler (Mic; BioMolecular Systems)
ABI Prism® 7500 SDS (Applied Biosystems)
QuantStudio™ 5 (Applied Biosystems)

Product No.: 870002, 870003

48 reactions, 96 reactions

Please refer to Storage and Shelf Life in this IFU

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Content

1. Intended Use

The LyteStar™ TB-MDR PCR Kit 1.0 is intended for the specific detection and differentiation of rifampicin and isoniazid resistance markers in *Mycobacterium tuberculosis* positive specimens from human sputum, bronchial washings, cerebrospinal fluid (CSF), urine, body fluids, tissue, and EDTA-whole blood. The LyteStar™ TB-MDR PCR Kit 1.0 is a dual-tube assay targeting the *rpoB* gene for the detection of rifampicin-resistant *M. tuberculosis* strains and the *katG* and *inhA* genes for the detection of isoniazid-resistant *M. tuberculosis* strains.

The LyteStar™ TB-MDR PCR Kit 1.0 is for professional use only.

2. Kit Components

Catalog no.	870002	870003
Master 1A	2 x 264 µl	2 x 528 µl
Master 1B	2 x 96 µl	2 x 192 µl
Master 2A	2 x 282 µl	2 x 564 µl
Master 2B	2 x 78 µl	2 x 156 µl
Internal Control (IC)	500 µl	800 µl
Positive Control (PC)	200 µl	400 µl
PCR grade water	250 µl	500 µl

3. Storage and Shelf Life

- The LyteStar™ TB-MDR PCR Kit 1.0 has a shelf life of 12 months from the manufacturing date.
- Store all PCR 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™ TB-MDR 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™ TB-MDR 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 troubleshooting.

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

Tuberculosis (TB) has re-emerged as the leading cause of infectious disease related death, surpassing COVID-19. In 2023, an estimated 8.2 million people were newly diagnosed with TB, representing the highest number recorded since the World Health Organization (WHO) began systematic surveillance in 1995 [1]. Tuberculosis spreads easily through airborne droplets, thus it continues to pose a major global health threat, particularly among individuals with a suppressed immune system such as HIV-positive individuals and those affected by drug resistant TB [1,2].

Effective TB management relies heavily on the early initiation of first-line antibiotics, primarily rifampicin and isoniazid. These drugs must be administered over several months under close supervision to ensure adherence and to prevent the emergence of resistance to rifampicin, isoniazid, or both, which is commonly known as multidrug-resistant TB (TB-MDR) [2]. TB-MDR often develops due to inappropriate

antibiotic use, suboptimal treatment regimens, or poor patient compliance. TB-MDR strains are transmissible and, if not detected and managed promptly, can lead to outbreaks within communities [3,4]. Thus far, approximately 450,000 individuals have developed TB-MDR worldwide [5], contributing substantially to global morbidity in TB-positive individuals.

Rapid and accurate detection of drug resistance is critical not only for the timely initiation of effective treatment but also for controlling the spread of resistant strains [6,7,8]. However, due to the slow-growing nature of *M. tuberculosis*, it takes several weeks to identify drug resistance via conventional culture-based drug susceptibility testing (DST). This delay can hinder on-time treatment decisions and increase the risk of disease progression and transmission. Additionally, phenotypic DST may not detect resistance in cases with heteroresistance or low-frequency mutations [9,10,11].

In order to address these diagnostic limitations, molecular methods such as real-time polymerase chain reaction (PCR) have become essential tools in the diagnosis of drug-resistant TB. These methods detect specific single-nucleotide polymorphism (SNP) associated with drug resistance, offering faster turnaround times and high diagnostic accuracy [12,13]. The LyteStar™ TB-MDR PCR Kit 1.0, was developed, using minor-groove binders (MGB), to target key mutations within the *rpoB* gene (rifampicin resistance) and *KatG* gene and *inhA* promoter region (isoniazid resistance) [9,14], to rapidly and reliably detect and differentiate rifampicin and isoniazid resistance TB-positive cases, enabling clinicians to initiate appropriate treatment regimes without delay. With high sensitivity and specificity, the kit supports timely intervention and improved patient outcomes, while also contributing to better TB control at the population level and supporting the global efforts to mitigate drug-resistant tuberculosis.

References:

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the detection of resistance to isoniazid and rifampicin: Policy update. https://www.who.int/publications-detail-redirect/9789241511261

9. Product Description

The LyteStar™ TB-MDR PCR Kit 1.0 is an *in vitro* diagnostic test system, based on real-time PCR technology for the qualitative detection and differentiation of rifampicin-resistant and isoniazid-resistant *Mycobacterium tuberculosis* specific DNA. The LyteStar™ TB-MDR PCR Kit 1.0 consists of a dual-tube assay targeting the RNA polymerase β subunit (*rpoB*) gene for the detection of rifampicin-resistant tuberculosis and the catalase-peroxidase enzyme (*katG*) gene and the *inhA* promoter region (enoyl-ACP reductase regulator) for isoniazid-resistant tuberculosis. The LyteStar™ TB-MDR PCR Kit 1.0 includes a heterologous amplification system (Internal Control) in tube 1, to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit. The Internal Control used in the LyteStar™ TB-MDR PCR Kit 1.0 is an artificial DNA sequence with no homology to any known genomes.

The LyteStar™ TB-MDR PCR Kit 1.0 utilizes real-time polymerase chain reaction (PCR) technology for the amplification of specific target sequences consisting of single-nucleotide polymorphism (SNP) or mismatches, and target specific minor-groove binder (MGB) probes for the detection of the amplified DNA. The mismatch-intolerant MGB probes are labelled with a fluorescent reporter and an Eclipse Dark Quencher (EDG) conjugated to the MGB moiety at the 3'end.

In the assay, probes specific for *rpoB* gene of rifampicin-resistant tuberculosis and the *katG* and *inhA* genes for isoniazid-resistant tuberculosis are labelled with the fluorophore FAM, and the probe specific for the Internal Control is labelled with the fluorophore HEX. The MGB probes for *rpoB* gene specifically detect the mutations in rifampicin-resistant *Mycobacterium tuberculosis* only, while the *katG* and *inhA* gene probes specifically detects the mutations in isoniazid-resistant *Mycobacterium tuberculosis* only, which allows differentiation between rifampicin- and isoniazid-resistant *Mycobacterium tuberculosis,* while excluding amplification of wild-type *Mycobacterium tuberculosis*. Using MGB probes linked to distinguishable dyes enables the parallel detection of rifampicin-resistant tuberculosis, isoniazid-resistant tuberculosis, and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides in the two assays were designed by mapping mutant *rpoB*, *katG* and *inhA* sequences to H37Rv reference sequence (NC_000962.3) using multiple sequence alignment, to identify single nucleotide mismatches or single nucleotide polymorphisms (SNPs).

The test consists of two processes in a dual-tube assay:

- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probe

The LyteStar™ TB-MDR PCR Kit 1.0 comprises of two separate reactions to detect rifampicin-resistant tuberculosis and isoniazid-resistant tuberculosis:

- Reaction Tube 1 is designed to detect rifampicin-resistant tuberculosis and Internal Control
- Reaction Tube 2 is designed to detect isoniazid-resistant tuberculosis

Reaction Tube	Detection	Reporter	Quencher
1	Rifampicin-resistant tuberculosis	FAM	MGB-Eclipse®
	Internal Control	HEX	IBFQ
2	Isoniazid-resistant tuberculosis	FAM	MGB-Eclipse®

The LyteStar™ TB-MDR PCR Kit 1.0 consists of:

- Four Master reagents - one Master A and one Master B for each Reaction Tube (Master 1A, Master 2A, Master 1B and Master 2B)
- 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 *rpoB* gene of rifampicin-resistant tuberculosis specific DNA, *katG* and *inhA* genes of isoniazid-resistant tuberculosis specific DNA, and Internal Control in two reaction setups.

The Positive Control (PC) contains synthesized target genes of rifampicin-resistant tuberculosis and isoniazid-resistant tuberculosis.

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

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

- Rotor-Gene Q5/6 Plex Platform (Qiagen)
- CFX96™ (BioRad)
- CFX Opus 96 (BioRad)
- Magnetic Induction Cycler (MIC; BioMolecular Systems)
- ABI Prism® 7500 SDS (Applied Biosystems)
- QuantStudio™ 5 (Applied Biosystems)

10. Material and Devices required but Not Provided

- Appropriate real-time PCR instrument
- 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
- Sterile, nuclease-free water
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material. **(White tubes are recommended. Do not use clear tubes)**.

11. Specimen Storage

- Suitable specimens include sputum, bronchial washings, cerebrospinal fluid (CSF), urine, tissue, and EDTA-whole blood specimens.
- 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/10656/66348

- Ministry of Health (MOH) (2023). Guidelines for the safe transport of clinical specimens and infectious substances in Malaysia 2023. https://imr.nih.gov.my/images/intranet/UPS/2024/GUIDELINES-FOR-THE-SAFE-TRANSPORT-OF-CLINICAL-SPECIMENS.pdf

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- Association of Public Health Laboratory (APHL). Specimen collection, handling, transport and processing. https://www.aphl.org/programs/infectious_disease/tuberculosis/TBCore/TB_Specimen_Collection_thru_Processing_TrainerNotes.pdf

- Centers for Disease Control and Prevention (CDC) (2024). Clinical and diagnosis guidelines for tuberculosis. https://www.cdc.gov/tb/hcp/testing-diagnosis/clinical-and-laboratory-diagnosis.html

12. Instructions for Use

12.1. Sample Preparation

Extracted DNA is the starting material for the LyteStar™ TB-MDR PCR Kit 1.0. The quality of the extracted DNA has a profound impact on the performance of the whole 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™ TB-MDR PCR Kit 1.0:

- LyteStar™ TB/NTM PCR Kit 3.1 - Extraction Reagents (AstronDX Technologies)
- SpinStar™ Total DNA Kit (AstronDX Technologies)

For detailed instructions regarding the extraction procedure using the SpinStar™

Total DNA Kit please contact our Technical Support (**16. Technical Support**).

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with the LyteStar™ TB-MDR 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 x 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 wash buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.

12.4. 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™ TB-MDR PCR Kit 1.0 contains a heterologous Internal Control (IC). The Internal Control is used ONLY as a PCR inhibition control when using the LyteStar™ TB/NTM PCR Kit 3.1 - Extraction Reagents. The IC can only be detected in Reaction Tube 1.

NOTE

For the boiling lysis extraction protocol using the LyteStar™ TB/NTM PCR Kit 3.1 - Extraction Reagents, the Internal Control is used as a PCR inhibition control ONLY and is added to the Master Mix.

(i) The Internal Control can be used as both (i) a PCR inhibition control and (ii) a control of the sample preparation procedure if a spin column-based nucleic acid extraction protocol is used.

(ii) **For the boiling lysis extraction protocol**, 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 1A	11 µl	132 µl
Master 1B	4 µl	48 µl
IC	0.5 µl	6 µl
Volume Master Mix	**15.5 µl**	**186 µl**

(iii) **For the spin column-based extraction protocol**, the IC may be used as a control for the sample preparation procedure and as a PCR inhibition control, therefore, the IC has to be added during the nucleic acid extraction procedure.

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 1A	11 µl	132 µl
Master 1B	4 µl	48 µl
Volume Master Mix	**15 µl**	**180 µl**

NOTE

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

3. For Reaction Tube 2, the Master Mix is set up according to the following pipette scheme:

Number of Reactions	1	12
Master 2A	11.75 µl	141 µl
Master 2B	3.25 µl	39 µl
Volume Master Mix	**15 µl**	**180 µl**

12.5. Reaction Setup

1. Pipette 15 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 xg (~3,000 rpm) for 30s.

Reaction Setup Tube 1	
Master Mix	15 µl
Sample or Control	5 µl
Total Volume	**20 µl**

Reaction Setup Tube 2	
Master Mix	15 µl
Sample or Control	5 µl
Total Volume	**20 µl**

13. Programming the Real-Time PCR Instrument

13.1 Settings

• Define the following settings:

Settings	
Reaction Volume	20 µl
Ramp Rate	Default
Passive Reference*	ROX

*Only for ABI7500 and QuantStudio 5

13.2 Fluorescent Detectors (Dyes)

• Define the following fluorescent detectors:

Reaction Tube	Detection	Detector Name	Reporter	Quencher
1	Rifampicin-resistant tuberculosis specific DNA (*rpoB* gene)	RIF	FAM	MGB-Eclipse®
	Internal Control	IC	HEX*	IBFQ
2	Isoniazid-resistant tuberculosis specific DNA (*katG* and *inhA* genes)	INH	FAM	MGB-Eclipse®

*Set VIC channel when using ABI7500 and QuantStudio 5

13.3 Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
UNG Reaction	Hold	1	-	35 °C	5:00 min
Initial Activation	Hold	1	-	95 °C	5:00 min
Denaturation	Cycling	45	-	95 °C	5 sec
Annealing			√	60 °C	20 sec
Extension			-	70 °C	30 sec
Final Extension	Hold	1	-	70 °C	3:00 min

√ Signal acquisition: activate FAM 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™ TB-MDR 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 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 *(RIF)*	Positive Control *(INH)*	Internal Control
Target CT value	< 35 cycles	< 35 cycles	≤ 45 cycles*

*Required for unknown samples that do not amplify in FAM channel

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 extracting the original samples again.

14.2 Interpretation of Results

Reaction Tube 1

Reaction Tube	FAM *RIF*	HEX *Internal Control*	Result Interpretation
1	+	+*	Rifampicin-resistant tuberculosis specific DNA detected. *Positive for Rifampicin-resistant tuberculosis*
	-	+	Rifampicin-resistant tuberculosis specific DNA not detected. The sample does not contain detectable amounts of Rifampicin-resistant tuberculosis specific DNA.
	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For Rifampicin-resistant tuberculosis *rpoB* gene (FAM 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 detection channels. A high tuberculosis load in the sample can lead to reduced or absent Internal Control signals.

Reaction Tube 2

Reaction Tube	FAM *INH*	HEX *Internal Control*	Result Interpretation
2	+	NA	Isoniazid-resistant tuberculosis specific DNA detected. *Positive for Isoniazid-resistant tuberculosis*
	-	+ (Refer to Tube 1)	Isoniazid-resistant tuberculosis specific DNA not detected. The sample does not contain detectable amounts of Isoniazid-resistant tuberculosis specific DNA.
	-	- (Refer to Tube 1)	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For Isoniazid-resistant tuberculosis *katG* and *inhA* gene (FAM channel) "+" refers to amplification curve detected at CT ≤ 45 cycles. "-" refers to no amplification / no CT obtained.

14.2.1 Analysis Settings for Cycler Software

Cycler	Settings to Apply
Rotor-Gene Q	After the run is completed, activate the "Outlier Removal" function in the FAM channel. Set the "Percentage of largest FI change" to "**5-10**%"
CFX96™ CFX Opus 96	After the run is completed: 1) Normalize Fluorescence signals in all channels by selecting "**Apply Fluorescence Drift Correction**" from the Baseline Setting menu 2) Adjust the "Cycles to Analyze" settings to "Analyze Data from Cycle: **8 to 45**"

14.2.2 Threshold Settings for Cycler Software

Cycler	Threshold		
	FAM		HEX
	RIF Channel	INH Channel	IC Channel
Rotor-Gene Q	0.05 norm. fluoro.		0.05 norm. fluoro.
CFX96™	100 RFU		100 RFU
CFX Opus 96	100 RFU		100 RFU
Mic qPCR	Auto		Auto
ABI7500	15,000 ΔRn		15,000 ΔRn
QuantStudio 5	50,000 ΔRn		50,000 ΔRn

14.2.2 CT Cut-Off Values for Clinical Samples

	FAM RIF Channel	FAM INH Channel
CT Cut-Off Value	< 45 cycles	< 45 cycles

15. Performance Evaluation

The analytical performance evaluation of the LyteStar™ TB-MDR PCR Kit 1.0 was accomplished using quantified rifampicin-resistant and isoniazid-resistant *M. tuberculosis* specific DNA.

15.1 Analytical Sensitivity

The analytical sensitivity (limit of detection: LoD) of the LyteStar™ TB-MDR PCR Kit 1.0 is defined as the concentration of rifampicin-resistant and isoniazid-resistant *M. tuberculosis* DNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined by analyzing rifampicin-resistant and isoniazid-resistant *M. tuberculosis* genomic DNA of known concentration.

A dilution series of AmpliRun® Total MTB RIF Resistant (Sputum) and AmpliRun® Total MTB INH Resistant (Sputum) was prepared by spiking into sputum and extracted with the LyteStar™ TB/NTM PCR Kit 3.1- Extraction Reagents. Extracted

rifampicin-resistant and isoniazid-resistant *M. tuberculosis* genomic DNA was analyzed with the LyteStar™ TB-MDR PCR Kit 1.0. Results analyzed by Probit analysis (Table 1 and Table 2).

The analytical sensitivity of the LyteStar™ TB-MDR PCR Kit 1.0 in consideration of a specific nucleic acid extraction method in combination with the CFX96™ platform (BioRad) was determined at 18.02 copies/µl for rifampicin-resistant *M. tuberculosis* target, and 39.67 copies/µl for isoniazid-resistant *M. tuberculosis* target.

Table 1. PCR results used for the calculation of the analytical sensitivity of rifampicin-resistant *M. tuberculosis* target for the LyteStar™ TB-MDR PCR Kit 1.0 in combination with the CFX96™ platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
200.0	12	12	100
63.3	12	12	100
20.0	12	12	100
6.33	12	8	66.67
2.00	12	1	8.33
0.633	12	1	8.33
0.200	12	0	0
0.063	12	0	0

Table 2. PCR results used for the calculation of the analytical sensitivity of isoniazid-resistant *M. tuberculosis* target for the LyteStar™ TB-MDR PCR Kit 1.0 in combination with the CFX96™ platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
350.0	12	12	100
221.52	12	12	100
110.76	12	12	100
35.05	12	11	91.67
11.09	12	2	16.67
3.51	12	0	0
1.11	12	0	0
0.35	12	0	0
0.11	12	0	0

15.2 Analytical Specificity

The analytical specificity of the LyteStar™ TB-MDR 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 the LyteStar™ TB-MDR PCR Kit 1.0 specifically detect only rifampicin-resistant *M. tuberculosis* and isoniazid-resistant *M. tuberculosis*.

The specificity of the LyteStar™ TB-MDR PCR Kit 1.0 was evaluated by testing genomic DNA extracted from other pathogens likely to be present in the same material as rifampicin-resistant and isoniazid-resistant *M. tuberculosis*, or that cause similar symptoms to these bacteria (Table 3).

Table 3. Microorganisms tested to demonstrate the analytical specificity of the LyteStar™ TB-MDR PCR Kit 1.0.

LyteStar™ TB-MDR PCR Kit 1.0			
Organisms	***RIF* (FAM channel)**	***INH* (FAM channel)**	***Internal Control* (HEX channel)**
Mycobacterium tuberculosis (Rifampicin resistant)	**positive**	negative	valid
Mycobacterium tuberculosis (Isoniazid resistant)	negative	**positive**	valid
Mycobacterium tuberculosis	negative	negative	valid
Mycobacterium bovis	negative	negative	valid
Mycobacterium abscessus	negative	negative	valid
Mycobacterium avium	negative	negative	valid
Mycobacterium gordonae	negative	negative	valid
Mycobacterium intracellulare	negative	negative	valid
Mycobacterium ulcerans	negative	negative	valid
Bordetella parapertussis	negative	negative	valid
Borrelia burgdorferi strain B31	negative	negative	valid
Chlamydophila pneumonia	negative	negative	valid
Haemophilus influenzae	negative	negative	valid
Klebsiella pneumoniae	negative	negative	valid
Legionella pneumophila	negative	negative	valid
Orientia tsutsugamushi	negative	negative	valid
Rickettsia conorii	negative	negative	valid
Rickettsia monacensis	negative	negative	valid
Human bocavirus	negative	negative	valid
Human herpesvirus 5 HCMV	negative	negative	valid
Human enterovirus 71	negative	negative	valid
Moraxella catarrhalis	negative	negative	valid
Mycoplasma pneumoniae	negative	negative	valid

The LyteStar™ TB-MDR PCR Kit 1.0 does 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™ TB-MDR PCR Kit 1.0	48	870002
LyteStar™ TB-MDR PCR Kit 1.0	96	870003
LyteStar™ TB/NTM PCR Kit 3.1	96	883103
SpinStar™ Total DNA Kit 2.0	100	821803

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