Instructions for Use

LyteStar™
2019-nCoV RT-PCR Kit 2.0

LyteStar™
2019-nCoV RT-PCR Kit 2.0

For detection of novel Coronavirus (SARS-CoV-2) from human specimens

For use with

Rotor-Gene Q5/6 plex Platform (Qiagen)
ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
QuantStudio™ 5 (Applied Biosystems)
CFx96™ (BioRad)
Magnetic Induction Cycler (Mic; Bio Molecular Systems)
ultraSBMS24 (MEDsan Biotech)

IVD For *in vitro* diagnostic use

REF Product No.: 888003

96 reactions

Please refer to Storage and Shelf Life in this IFU

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Content

1. Intended Use

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is intended for the specific detection of SARS-CoV-2 RNA in human respiratory specimens (bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal and oropharyngeal swabs placed in VTM, nasopharyngeal wash/aspirate, and nasal wash/aspirate). The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is a dual target assay comprising a screening assay targeting the *E* gene and a confirmation assay targeting the *RdRP* gene.

Besides compatible extraction systems, the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 has been validated for use with a direct PCR protocol, without prior nucleic acid extraction. The direct PCR protocol has been validated for single samples and pools of up to 5 individual samples in VTM. **Refer to 12.1.2 Sample Preparation via Direct PCR Protocol.**

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is for professional use only. The direct PCR protocol has been validated only for Viral Transport Media (VTM) that do NOT contain Guanidium Thiocyanate. **Refer to 9. Product Description for Validated VTM**.

NOTE

⚠ ***Viral Transport Media (VTM) containing Guanidinium Thiocyanate are NOT suitable for direct PCR protocols.***

2. Kit Components

Catalog no.	888003
Master A	2 x 312 µl
Master B	4 x 324 µl
Internal Control (IC)	800 µl
Positive Control (PC)	200 µl
PCR grade water	500 µl

3. Storage and Shelf Life

- The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 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™ 2019-nCoV RT-PCR Kit 2.0 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™ 2019-nCoV RT-PCR Kit 2.0 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

Coronaviruses are a group of enveloped viruses with a positive-sense, single-stranded RNA genome. There are six human Coronaviruses that cause illness ranging from common cold to more severe disease such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

The novel Coronavirus SARS-CoV-2, which causes the outbreak of pneumonia cases in Wuhan City, Hubei Province of China since late December 2019, has been identified as a new (seventh) type of Coronavirus in January 2020 causing pneumonia. The virus belongs to the genus Betacoronavirus and is closely related to bat-SARS-like Coronavirus, but genetically distinct / divergent from SARS-CoV and MERS-CoV [1]. Thus, SARS-CoV-2 is thought to be originated from bats and spread by animal-to-human transmission, via yet unknown intermediate animal host/s. Reports of infection among healthcare workers and family members who are in close contact with SARS-CoV-2-infected patients, also indicated human-to-human transmission.

On the 30th of January 2020, WHO declared the novel coronavirus outbreak a Public Health Emergency of International Concern (PHEIC). As of 20th February 2022, more than 418,650,474 confirmed cases and 5,858,224 deaths were reported to WHO [2]. Symptoms include fever, coughs and shortness of breath; with an incubation period of 1-14 days. In severe cases, the infection can cause pneumonia, acute respiratory distress syndrome, kidney failure and death. Since 2020, the development of a vaccine for disease caused by SARS-CoV-2 infection was expediated via unprecedented collaboration in the multinational pharmaceutical industry and governments. As of 14th February 2022, a total of 10,279,668,555 vaccine doses have been administered [2].

Various real-time RT-PCR assays have been published to detect SARS-CoV-2. The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 was developed based on two assays previously described [3,4]. One assay targets the *E* gene (Screening assay), and the second assay targets the *RdRP* gene (Confirmatory assay).

[1] Lu R, *et al*. Genomic characterisation and epidemiology of 2019 novel coronavirus:implications for virus origins and receptor binding. *Lancet*; published online January 29, 2020 https://doi.org/10.1016/S0140-6736(20)30251-8.

[2] Novel Coronavirus (2019-nCoV), WHO Situation Report – 13.

[3] Corman V, Bleicker T, Brünink S, Drosten C. Diagnostic detection of 2019-nCoV by real-time RT-PCR. Protocol and preliminary evaluation Jan 17th, 2020.

[4] Chan JF-W, *et al*. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse Transciption PCR assay validated *in vitro* and with clinical specimens. (2020) *Journal of Clinical Microbiology*. (58) e003:10-20.

9. Product Description

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is an *in vitro* diagnostic test system, based on real-time PCR technology, for the qualitative detection of novel coronavirus (SARS-CoV-2) specific RNA.

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 consists of two assays, one targeting the *E* gene and the other targeting RNA-dependent RNA polymerase gene (*RdRP*) of the SARS-CoV-2 genome. The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit. The Internal Control template used in the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is an *in-vitro* transcribed RNA of an artificial sequence with no homology to any known genomes.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

In the assays, probes specific for *E* gene of SARS-CoV-2 RNA are labeled with the fluorophore FAM™, and probes specific for *RdRP* gene of SARS-CoV-2 RNA are labeled with fluorophore Cy5. The *E* gene probe detects members of subgenus Sarbecovirus of the genus Betacoronavirus (which includes SARS-CoV-2, SARS-CoV and bat-SARS-related CoVs). The *RdRP* gene probe is specific to SARS-CoV-2 only. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore HEX™. Using probes linked to distinguishable dyes enables the parallel detection of SARS-CoV-2 specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides included in the two assays were published by Victor Corman *et al*. [3], and Jasper Fuk-Woo Chan *et al*. [4].

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

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 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 reverse transcription, amplification and target detection of *E* gene of Sarbecovirus specific RNA (including SARS-CoV-2), *RdRP* gene (SARS-CoV-2 specific), and Internal Control in one reaction setup.

The Positive Control (PC) contains *in vitro* transcripts of synthesized target genes of SARS-CoV-2.

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 was developed and validated to be used with the following real-time PCR instruments:

- Rotor-Gene Q 5/6 plex Platform (Qiagen)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- QuantStudio™ 5 (Applied Biosystems)
- CFx96™ (BioRad)
- Magnetic Induction Cycler (Mic; Bio Molecular Systems)
- ultraSBMS24 (MEDsan Biotech)

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is compatible for use with a direct PCR protocol, without prior nucleic acid extraction. The direct PCR protocol to be used with the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 has been validated to be used with the following viral transport media:

- Viral Transport Medium:
  TRANSPORT MEDIUM - 2 ml - 16.5x57 mm Tube - No swab included
  (Vircell, Cat. No. TM005)

- Inactivating Viral Transport Medium:
  PD VTM with nasal & throat swab, 3 ml inactivated medium, 10 ml tube
  (Premier Diagnostics, Cat. No. 8011900)

- Non-Inactivating Viral Transport Medium:
  PD VTM with nasal & throat swab, 3 ml non-inactivated medium, 10 ml tube
  (Premier Diagnostics, Cat. No. 8011903)

NOTE

Viral Transport Media (VTM) containing Guanidinium Thiocyanate are NOT suitable for direct PCR protocols.

The direct PCR protocol to be used with the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 was developed and validated to be used with the following real-time PCR instrument:

- Magnetic Induction Cycler (Mic; Bio Molecular Systems)

The direct PCR protocol has been validated for single samples and for pools of up to 5 individual samples for a pooling strategy whereby five swab samples are collected in a single vial of Viral Transport Medium (VTM).

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 48-well or 96-well reaction plates or reaction tubes with corresponding (optical) closing material

11. Specimen Storage

- Suitable specimens include bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal and oropharyngeal swabs (placed in the same VTM), nasopharyngeal wash/aspirate, and nasal wash/aspirate.

- Follow specimen transport and storage conditions outlined in the following guidelines:
  - WHO Interim Guidance on Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases, version 19 Mar 2020.
  - CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for Covid 19 Testing, version 25 Oct. 2021.

12. Instructions for Use

12.1. Sample Preparation

12.1.1. Sample Preparation via Nucleic Acid Extraction System

Extracted RNA is the starting material for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0. The quality of the extracted RNA 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™ 2019-nCoV RT-PCR Kit 2.0:

- SpinStar™ Viral Nucleic Acid Kit (AstronDX Technologies)
- QIAamp® MinElute Virus Spin Kit (Qiagen)
- QIAamp® Viral RNA Mini Kit (Qiagen)
- HighPure® Viral Nucleic Acid Kit (Roche)
- QIAsymphony® (Qiagen)
- NucliSENS® easyMag (bioMérieux)
- MagNA Pure 96 System (Roche)
- MagCore® Plus II Automated Nucleic Acid Extractor (RBC Bioscience)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with LyteStar™ 2019-nCoV RT-PCR Kit 2.0 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 washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.

12.1.2. Sample Preparation via Direct PCR Protocol

Swab samples collected in VTM is the starting material for the direct PCR protocol to be used with LyteStar™ 2019-nCoV RT-PCR Kit 2.0.

The following direct PCR protocols are suitable for use with the LyteStar™ 2019-nCoV RT-PCR Kit 2.0.

Protocol 1: For Viral Transport Medium (VTM)

1) Mix the specimen collected in the Viral Transport Medium (VTM) by vortexing or pipetting.

2) Dilute the sample in a 1 : 2 ratio with nuclease free-water (200 µl specimen diluted in 400 µl nuclease-free water) in a 1.5ml Eppendorf tube.

3) Pulse vortex the tube for 15 seconds, and briefly centrifuge.

4) Transfer the tube into a dry bath (heat-block), pre-heated to 95°C, for 4 minutes. (This step may be omitted when using Inactivating VTMs).

5) Mix the tube gently by tapping or vortexing, and centrifuge at 10,000 rpm for 1 minute.

6) Use 5 µl of the sample as PCR template and continue with Master Mix Setup.

NOTE

The heat inactivation step (Step 4) may be omitted when using Inactivating Viral Transport Medium

Protocol 2: For LyteStar™ Direct PCR Sample Medium

1) Mix the specimen collected in the LyteStar™ Direct PCR Sample Medium by tapping, vortexing or pipetting.

2) Use 5 µl of the sample as PCR template and continue with Master Mix Setup.

12.1.3. Sample Preparation for Pooled-Sampling Strategy

A pooled-sampling strategy of 5 swab samples collected in a single vial of Viral Transport Medium (VTM) has been validated for use with the Direct PCR protocol.

1) Collect 5 swab samples in a single vial of Viral Transport Medium (VTM).

2) When using validated Viral Transport Medium (VTM) continue with **Protocol 1**; when using the LyteStar™ Direct PCR VTM continue with **Protocol 2 (12.1.2. Sample Preparation via Direct PCR Protocol).**

3) Use 5 µl of the sample as PCR template and continue with Master Mix Setup.

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™ 2019-nCoV RT-PCR Kit 2.0 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.

NOTE

⚠ ***For the direct PCR protocol, the Internal Control is used as a PCR inhibition control ONLY and is added to the Master Mix***

(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	6.5 µl	78 µl
Master B	13.5 µl	162 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 per sample 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	6.5 µl	78 µl
Master B	13.5 µl	162 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 48-/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 48-/96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate caps.

6. Centrifuge the 48-/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
Passive reference*	None

*Only for ABI7500 and QuantStudio 5

13.2 Fluorescent Detectors (Dyes)

• Define the following fluorescent detectors:

Detection	Detector Name	Reporter	Quencher
Sarbecovirus (*E* gene) specific RNA	E	FAM	BHQ 1
SARS-CoV-2 (*RdRP* gene) specific RNA	RdRP	Cy5	BHQ 1
Internal Control	IC	HEX	BHQ 1

13.3 Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse-transcription	Hold	1	-	50 °C	10:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	5 sec
			√	55 °C	30 sec

√ Signal acquisition: activate FAM, HEX, and Cy5 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™ 2019-nCoV RT-PCR Kit 2.0 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 *(E gene)*	Positive Control *(RdRP gene)*	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 *E gene*	Cy5 *RdRP gene*	HEX *Internal Control*	Result Interpretation
+	+	+*	Sarbecovirus *E* and SARS-CoV-2 *RdRP* specific RNA detected. *Positive for SARS-CoV-2*
+	-	+*	Sarbecovirus *E* specific RNA detected. SARS-CoV-2 *RdRP* specific RNA not detected. *Presumptive positive for SARS-CoV-2*[A,B]
-	+	+*	Sarbecovirus *E* specific RNA not detected. SARS-CoV-2 *RdRP* specific RNA detected. *Presumptive positive for SARS-CoV-2*[A,B]
-	-	+	Both Sarbecovirus *E* and SARS-CoV-2 *RdRP* specific RNA not detected. The sample does not contain detectable amounts of SARS-CoV-2 specific RNA.
-	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For *E* gene (FAM channel) and *RdRP* 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 SARS-CoV-2 load in the sample can lead to reduced or absent Internal Control signals.

[A] Detection of either *E* gene or *RdRP* gene alone might be due to low viral RNA concentration close to the limit of detection of the respective genes. Specifically, detection of *E* gene alone is frequently observed and may be attributed to the unique CoV transcription pattern in which subgenomic RNAs take part in active viral replication, resulting in higher copy numbers for *E* gene.

[B] Sample may be repeated from extraction. If the repeat result remains *E* positive or *RdRP* positive only, then additional confirmatory assay may be needed.

14.2.1 Threshold Settings for Cycler Software

Cycler	Threshold		
	E channel	*RdRP* channel	IC channel
Rotor-Gene	0.05 norm. fluoro	0.05 norm. fluoro	0.05 norm. fluoro
CFX96	100 RFU	100 RFU	100 RFU
ABI7500	15,000 ΔRn	15,000 ΔRn	5,000 ΔRn
QuantStudio 5	50,000 ΔRn	25,000 ΔRn	10,000 ΔRn
Mic qPCR	Auto	Auto	Auto

14.2.2 CT Cut-Off Values for Clinical Samples

	FAM *E* Channel	Cy5 *RdRP* Channel
CT Cut-Off Value	< 45 cycles	< 45 cycles

15. Performance Evaluation

The analytical performance evaluation of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 was accomplished using quantified SARS-CoV-2 specific RNA.

15.1 Analytical Sensitivity

The analytical sensitivity (limit of detection:LoD) of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 is defined as the concentration of SARS-CoV-2 RNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined in consideration of a selected nucleic acid extraction method and the direct PCR protocol, by analyzing samples with known SARS-CoV-2 concentration.

15.1.1 In consideration of a Nucleic Acid Extraction Procedure

A dilution series of the Amplirun® Total SARS-CoV-2 Control (swab) was prepared by spiking into TE buffer and extracted with the SpinStar™ Viral Nucleic Acid Extraction Kit. Extracted SARS-CoV-2 RNA was analyzed with LyteStar™ 2019-nCoV RT-PCR Kit 2.0. Results were analyzed by Probit analysis (Table 1 and Table 2).

Nucleic Acid Extraction Procedure:

SpinStar™ Viral Nucleic Acid Extraction Kit 1.0 (AstronDX Technologies)
Sample volume: 200 µl
Elution volume: 60 µl

The analytical sensitivity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of SpinStar™ nucleic acid extraction method was determined at 2.14 copies/µl for *E* gene target and 1.68 copies/µl for *RdRP* gene target ($p \leq 0.05$).

Table 1. PCR results used for the calculation of the analytical sensitivity of *E* gene target for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of a particular extraction method and 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	12	12	100
6.3	12	12	100
2	12	12	100
0.63	12	10	83.3
0.2	12	6	50
0.063	12	2	16.7
0.02	12	0	0
0.0063	12	2	16.7
0.002	12	0	0
0.00063	12	0	0

Table 2. PCR results used for the calculation of the analytical sensitivity of *RdRP* gene target for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of a particular extraction method and 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	12	12	100
6.3	12	12	100
2	12	12	100
0.63	12	9	75
0.2	12	6	50
0.063	12	2	16.7
0.02	12	1	8.3
0.0063	12	0	0
0.002	12	0	0
0.00063	12	0	0

15.1.2 In consideration of a Direct PCR Protocol

A dilution series of the Amplirun® Total SARS-CoV-2 Control (swab) was also prepared by spiking into either the Viral Transport Medium or the LyteStar™ Direct PCR Sample Medium and undergoing the sample preparation via the direct PCR protocol (**12.1.2 Sample Preparation via Direct PCR Protocol**). SARS-CoV-2 RNA was analyzed with LyteStar™ 2019-nCoV RT-PCR Kit 2.0. Results were analyzed by Probit analysis.

The analytical sensitivity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0, when used with the Viral Transport Media, using Protocol 1 (**12.1.2 Sample Preparation via Direct PCR Protocol**) was determined at 5538.1 copies/ml (5.38 copies/µl) for *E* gene target and 7518.2 copies/ml (7.52 copies/µl) for *RdRP* gene target ($p \leq 0.05$) (Table 3 and Table 4).

The analytical sensitivity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0, when used with the LyteStar™ Direct PCR Sample Medium, using Protocol 2 (**12.1.2 Sample Preparation via Direct PCR Protocol**), was determined at 2028.4 copies/ml (2.03 copies/µl) for *E* gene target and 2080.7 copies/ml (2.08 copies/µl) for *RdRP* gene target ($p \leq 0.05$) (Table 5 and Table 6).

Table 3. PCR results used for the calculation of the analytical sensitivity of *E* gene target for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of Protocol 1 (**12.1.2 Sample Preparation via Direct PCR Protocol**) using Viral Transport Media, and in combination with the Magnetic Induction Cycler (Mic; Bio Molecular Systems).

Concentration (copies/ml)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
10,000	12	12	100
5000	12	12	100
3164.4	12	10	83.3
1001.4	12	7	58.3
316.9	12	1	8.3
100.3	12	2	16.7
31.7	12	0	0
10.0	12	0	0
3.2	12	0	0

Table 4. PCR results used for the calculation of the analytical sensitivity of *RdRP* gene target for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of Protocol 1 (**12.1.2 Sample Preparation via Direct PCR Protocol**) using Viral Transport Media, and in combination with the Magnetic Induction Cycler (Mic; Bio Molecular Systems).

Concentration (copies/ml)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
10,000	12	12	100
5000	12	12	100
3164.4	12	9	75.0
1001.4	12	3	25.0
316.9	12	1	8.3
100.3	12	1	8.3
31.7	12	0	0
10.0	12	0	0
3.2	12	0	0

Table 5. PCR results used for the calculation of the analytical sensitivity of *E* gene target for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of Protocol 2 (**12.1.2 Sample Preparation via Direct PCR Protocol**) using LyteStar™ Direct PCR Media, and in combination with the Magnetic Induction Cycler (Mic; Bio Molecular Systems).

Concentration (copies/ml)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
10,000	12	12	100
5000	12	12	100
3164.4	12	12	100
1001.4	12	10	83.3
316.9	12	3	25.0
100.3	12	2	16.7
31.7	12	0	0
10.0	12	0	0
3.2	12	0	0

Table 6. PCR results used for the calculation of the analytical sensitivity of *RdRP* gene target for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of Protocol 2 (**12.1.2 Sample Preparation via Direct PCR Protocol**) using LyteStar™ Direct PCR Media, and in combination with the Magnetic Induction Cycler (Mic; Bio Molecular Systems).

Concentration (copies/ml)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
10,000	12	12	100
5000	12	12	100
3164.4	12	12	100
1001.4	12	8	66.7
316.9	12	6	50.0
100.3	12	0	16.7
31.7	12	0	0
10.0	12	0	0
3.2	12	0	0

15.1.3 Equivalence of a Pooled Sampling Strategy

For pools of up to 5 individual samples per pool, a pooling strategy was adopted whereby 5 swabs are collected in one single vial of Viral Transport Medium (VTM).

To validate performance of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 for pooled samples, a set of 2 swabs each were spiked with identical (10xLoD or 5xLoD) concentrations of Amplirun® total SARS-CoV-2 Control (swab). One swab of each set was processed as a single sample in its own vial of VTM, while the second swab of each set was processed in a pool of 5 swabs together with 4 negative swabs in a single vial of VTM. Detection of positive swabs in a pool of 5 samples is equivalent to the detection of positive swabs tested alone. Results are summarized in Table 7.

Table 7. PCR results for *E* gene and *RdRP* gene amplification for the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 in consideration of a pooled-sampling strategy and in combination with the Magnetic Induction Cycler (Mic; Bio Molecular Systems).

Sample	Concentration	Number of samples tested	Number of samples positive		Hit Rate
			E gene	*RdRP* gene	
Single Swab	5 x LoD	6	6	6	100%
	10 x LoD	6	6	6	100%
Pooled Swabs	5 x LoD	6	6	6	100%
	10 x LoD	6	6	6	100%

15.2 Analytical Specificity

The analytical specificity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 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™ 2019-nCoV RT-PCR Kit 2.0 specifically detect SARS-CoV-2.

The specificity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 was evaluated by testing genomic RNA/DNA extracted from other pathogens likely to be present in the same sample material as SARS-CoV-2 virus, or that cause similar symptoms to the SARS-CoV-2 virus (Table 8). The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 did not cross-react with any of the specified organisms.

Table 8. Microorganisms tested to demonstrate the analytical specificity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 Kit.

LyteStar™ 2019-nCoV RT-PCR 2.0			
Organisms	***E gene (FAM channel)***	***RdRP gene (Cy5 channel)***	***Internal Control (HEX channel)***
Human metapneumovirus	negative	negative	valid
Enterovirus 68	negative	negative	valid
Human respiratory syncytial virus	negative	negative	valid
Human rhinovirus 77	negative	negative	valid
Human parainfluenza virus 1	negative	negative	valid
Human adenovirus 1	negative	negative	valid
Human coronavirus OC43	negative	negative	valid
Human coronavirus NL63	negative	negative	valid
Human coronavirus HKU1	negative	negative	valid
Human coronavirus 229E	negative	negative	valid
Influenza virus A, H1N1	negative	negative	valid
Influenza virus A, H3N2	negative	negative	valid
Influenza virus A, H5N6	negative	negative	valid
Influenza virus A, H7N9	negative	negative	valid
Influenza virus A, H9N2	negative	negative	valid
Influenza virus B, Victoria lineage	negative	negative	valid
Influenza virus B, Yamagata lineage	negative	negative	valid
Haemophilus influenza	negative	negative	valid
Legionella pneumophila subsp. *Pneumophila*	negative	negative	valid
Mycobacterium tuberculosis	negative	negative	valid
Streptococcus pneumoniae	negative	negative	valid
Streptococcus pyogenes	negative	negative	valid
Bordetella pertussis	negative	negative	valid
Mycoplasma pneumoniae	negative	negative	valid
Chlamydophila pneumoniae	negative	negative	valid

15.3 Diagnostic Sensitivity and Specificity

The clinical performance of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0, in regards to diagnostic sensitivity and specificity, was evaluated through a retrospective study performed at a public medical university. The diagnostic sensitivity and specificity of the LyteStar™ 2019-nCoV RT-PCR Kit 2.0 was compared to the clinical site's reference method.

The LyteStar™ 2019-nCoV RT-PCR Kit 2.0 achieved a diagnostic sensitivity of 100% and diagnostic specificity of 100% for the detection of SARS-CoV-2.

		Reference Method	
		SARS-CoV-2 Positive (n=121)	**SARS-CoV-2 Negative (n=102)**
LyteStar™ 2019 nCoV RT-PCR Kit 2.0	**Detected**	121	0
	Not Detected	0	102
	Total	**121**	**102**
Overall Concordance		**100 %** 121 / 121 Sensitivity	**100 %** 102 / 102 Specificity

To calculate the diagnostic sensitivity of LyteStar™ 2019-nCoV RT-PCR Kit 2.0:

$$\text{Diagnostic Sensitivity [\%]} = \frac{\text{“correct positive”}}{\text{(“correct positive” + “false positive”)}} \times 100$$

To calculate the diagnostic specificity of LyteStar™ 2019-nCoV RT-PCR Kit 2.0:

$$\text{Diagnostic Specificity [\%]} = \frac{\text{“correct negative”}}{\text{(“correct negative” + “false negative”)}} \times 100$$

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
	*In vitro* diagnostic medical device
	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™ 2019-nCoV RT-PCR Kit 2.0	96	888003
SpinStar™ Viral Nucleic Acid Extraction Kit 1.0	100	811803
MagCore® Viral Nucleic Acid Extraction Kit, High Sensitivity (200µl/400µl), CART CODE 203	96	MVN400-06

NOTES

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