LyteStar™
2019-nCoV RT-PCR Kit 1.0

05/2020

Attention ! New version manual

Change in the content of this Instruction Manual (version 05/2020):

Section	Changes applied	Page no.
All sections	Virus name "2019-nCoV" replaced by "**SARS-CoV-2**". Kit name maintained as "LyteStar™ 2019-nCoV RT-PCR Kit 1.0"	-
3. Storage and Shelf Life	Inserted a note on handling of Master A (avoid vigorous vortex mixing)	4
13.1 Settings	Passive reference set as "**None**"	13
14.1.2 CT cut-off of PC and IC	CT cut-off for Internal Control changed from "< 37 cycles" to "**≤ 40** cycles"	15
	Table of CT cut-off inserted with new footnote, to avoid misinterpretation of PC CT cut-off as the diagnostic cut-off for clinical samples	
14.2 Interpretation of Results	A new footnote inserted to the table of result interpretation, explaining "+" and "-" symbols used for detection and non-detection of SARS-CoV-2 in clinical samples	16
	Threshold settings for E, RdRP and IC channels in RGQ, CFX96 and ABI7500 included in new sub-section 14.2.1	17

LyteStar™
2019-nCoV RT-PCR Kit 1.0

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

For use with

Rotor-Gene™ 3000/6000 (Corbett Research)
Rotor-Gene Q5/6 plex Platform (Qiagen)
ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
CFx96™ (BioRad)

RUO For Research Use Only

REF Product No.: 888002

48 reactions

Please refer to Storage and Shelf Life in this handbook

April 2020

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Content

1. Intended Use

The LyteStar™ 2019-nCoV RT-PCR Kit 1.0 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 1.0 is a dual target assay comprising a screening assay targeting the *E gene* and a confirmation assay targeting the *RdRP gene*.

2. Kit Components

Catalog no.	**888002**
User Manual	1
Master A	2 x 96 µl
Master B – target *E* gene	4 x 216 µl
Master B – target *RdRP* gene	4 x 216 µl
Internal Control (IC)	1000 µl
Positive Control (PC) – target *E* gene	150 µl
Positive Control (PC) – target *RdRP* gene	150 µl
PCR grade water	500 µl

3. Storage and Shelf Life

- The LyteStar™ 2019-nCoV RT-PCR Kit 1.0 has a shelf life of 6 months from the manufacturing date.
- Store all reagents at -20°C upon arrival.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Avoid vigorous vortex mixing of Master A, which contains RT enzyme.
- 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

Each lot of the LyteStar™ 2019-nCoV RT-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

ADT Biotech guarantees the performance of the LyteStar™ 2019-nCoV RT-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 ADT Biotech Technical Support (15. Technical Support) for troubleshooting.

ADT Biotech 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.

As of 2nd Feb 2020, more than 14,000 confirmed cases and 300 deaths were reported [2], with a vast majority of the confirmed cases (and all death cases) in China. WHO has since declared a Public Health Emergency of International Concern (PHEIC). Currently, there are no vaccines or treatment drugs available for disease caused by SARS-CoV-2 infection. 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.

Various real-time RT-PCR assays have been published to detect SARS-CoV-2. The LyteStar™ 2019-nCoV RT-PCR Kit 1.0 was developed based on two assays described [3]. 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.

9. Product Description

The LyteStar™ 2019-nCoV RT-PCR Kit 1.0 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 1.0 consists of two independent assays, one targeting the *E* gene and the other targeting RNA-dependent RNA

polymerase gene (*RdRP*) of the SARS-CoV-2 genome. The *E* gene assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

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 both assays, probes specific for *E* gene and *RdRP* gene of SARS-CoV-2 RNA are labeled with the fluorophore FAM™. 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].

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 1.0 consists of:

- Three Master reagents
  - Master A for target *E* gene and *RdRP* gene
  - Master B for target *E* gene
  - Master B for target *RdRP* gene
- Internal Control (IC)

- Two Positive Controls (PC)
  - PC for *E* gene
  - PC for *RdRP* gene
- 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) + Internal Control in one reaction and *RdRP* gene (SARS-CoV-2 specific) only in another reaction.

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

The LyteStar™ 2019-nCoV RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (Qiagen)
- ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems)
- CFx96 (BioRad)

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 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 2 Mar 2020.
  - CDC Interim guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-2019), version 14 Feb 2020.

12. Instructions for Use

12.1. Sample Preparation

Extracted RNA is the starting material for the LyteStar™ 2019-nCoV RT-PCR Kit 1.0. 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 1.0:

- SpinStar™ Viral Nucleic Acid Kit (ADT Biotech)
- 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)

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 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 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™ 2019-nCoV RT-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	2 µl	24 µl
Master B (*E* or *RdRP*)	18 µl	216 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	2 µl	24 µl
Master B (*E* or *RdRP*)	18 µl	216 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 for *E* gene / *RdRP* gene; 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	
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

*Not required for Rotor-Gene and CFX96 cyclers

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	FAM	BHQ1
Internal Control	IC	HEX*	BHQ1

* Set VIC channel when using ABI Prism 7500 SDS / 7500 Fast SDS

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	30:00 min
Denaturation	Hold	1	-	95 °C	15:00 min
Amplification	Cycling	45	-	95 °C	0:15 min
			√	58 °C	0:45 min

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 1.0 on different real-time PCR instruments please contact our Technical Support (15. 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 (*E* and *RdRP*)	POSITIVE	POSITIVE*
Negative Control	NEGATIVE	POSITIVE*

*IC amplification in *E* gene master mix only.

14.1.2 CT cut-off of PC and IC

	Positive Control (*E* gene)	Positive Control (*RdRP* gene)	Internal Control
CT cut-off	< 35 cycles	< 35 cycles	≤ 40 cycles*

*required for unknown samples without *E* and *RdRP* amplification in FAM channel

Note: The above CT cut-off 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 CT cut-off 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	HEX *E* gene	FAM *RdRP* gene	HEX *RdRP* gene	Result Interpretation
+	+*	+	NA	Sarbecovirus *E* and SARS-CoV-2 *RdRP* specific RNA detected. Send sample to national reference laboratory for confirmatory testing**
+	+*	-	NA	Sarbecovirus *E* specific RNA detected. SARS-CoV-2 *RdRP* specific RNA not detected. Repeat testing. Send sample to national reference laboratory for confirmatory testing**
-	+	+	NA	Sarbecovirus *E* specific RNA not detected. SARS-CoV-2 *RdRP* specific RNA detected. Repeat testing. Send sample to national reference laboratory for confirmatory testing**
-	+	-	NA	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.
-	-	-	NA	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

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

NA: not applicable

** According to national guidelines of respective countries

14.2.1 Threshold Settings for Cycler Software

Cycler	Threshold	
	***E / RdRP* channel**	**IC channel**
Rotor-Gene	0.10 norm. fluoro	0.05 norm. fluoro
CFX96	200 RFU	100 RFU
ABI7500	25,000 ΔRn	5,000 ΔRn

15. Technical Support

For customer support, please contact our Technical Support:

e-mail: techsupport@adt-biotech.com
phone: +603 7931 6760

16. Appendix

Explanation of Symbols

Product Number

Batch Code


Manufacturer


Contains sufficient for “n” tests/rxns


Temperature limitation


Version

17. Ordering Information

Products	**Packing** (reactions)	**Order No.**
LyteStar™ 2019-nCoV RT-PCR Kit 1.0	48	888002
LyteStar™ SARS-CoV-2 RT-PCR Kit 1.0 S	96	888103
Spinstar™ Viral Nucleic Acid Kit 1.0	100	811803

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