GSD NovaPrime®
SARS-CoV-2 (COVID-19)
RT-PCR

CE

For in-vitro diagnostic use only

Product Number:	PCOV6033	(96 Determinations)

CONTENTS

CONTENTS 2

ENGLISH 3

1. INTRODUCTION 3
2. INTENDED USE 3
3. PRINCIPLE OF THE ASSAY 3
4. MATERIALS PROVIDED 4
5. STABILITY AND STORAGE 4
   - **5.1.** Materials and Equipment needed, but not provided 4
6. SAMPLE COLLECTION AND PREPARATION 4
7. ASSAY PROCEDURE 4
   - **7.1.** Reaction Setup 4
   - **7.2.** Programming the Real-Time PCR Instruments 5
8. RESULTS 5

   Run Validation Criteria 6
   - **8.1.** Interpretation of Results 6
9. SPECIFIC PERFORMANCE CHARACTERISTICS 6
   - **9.1.** Precision 7
   - **9.2.** Limit of Detection (LoD) 7
   - **9.3.** Inclusivity 7
   - **9.4.** Cross-Reactivity 7
   - **9.5.** Negative and Positive Percent Agreement (NPA, PPA) 7
10. QUALITY CONTROL 7
11. TRADEMARKS AND DISCLAIMERS 7
12. PRECAUTIONS AND WARNINGS 8
    - **12.1.** Disposal Considerations 8
13. ORDERING INFORMATION 8

Bibliography 11

Symbols Key 12

ENGLISH

1. INTRODUCTION

End of 2019, a novel respiratory disease emerged in the city of Wuhan, Hubei Province of the People's Republic of China, and soon spread rapidly within the country and worldwide. The causative agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 (2019-nCoV), like the closely related SARS coronavirus (SARS-CoV), belongs to the genus Betacoronavirus within the family of coronaviruses. The zoonotic reservoir of the virus appears to be bats.

Coronaviruses are enveloped, positive single-stranded large RNA viruses that infect humans, but also a wide range of animals. The common human coronaviruses NL63, 229E, OC43 and HKU1 are widespread especially throughout the winter months. They are responsible for up to one third of all acute respiratory diseases, typically with mild symptoms (common cold). More than 80 % of the adult population have antibodies against human coronaviruses. The immunity from previous infections lasts only for a short period of time. Therefore, reinfections with the same pathogen are possible just after one year.

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected patients. In theory, smear infection and infection through the conjunctiva of the eyes are also possible.
The incubation period is in the median 5–6 days (and up to 14 days maximum).

The clinical manifestations of SARS-CoV-2-related COVID-19 disease include fever, cough, respiratory problems and fatigue. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates.

The initial clinical sign of COVID-19 which allowed case detection was pneumonia. But it turned out that the course of the disease is non-specific and varies widely, from asymptomatic courses to severe pneumonia with lung failure and death. However, based on current knowledge, around 80 % of the illnesses are mild to moderate.

Although severe courses of the disease also occur in younger patients and people without previous illness, the following groups of people have an increased risk of serious forms of the disease: elderly people (with a steadily increasing risk from around 50-60 years of age), smokers and people with certain diseases of the cardiovascular system or the lungs, patients with chronic liver diseases, diabetes mellitus, cancer, or patients with a weakened immune system (e.g. due to immune deficiencies or by taking drugs that suppress the immune system).

Currently, there is no specific treatment or vaccine available against SARS-CoV-2 infection.

Species	Disease	Symptoms e.g.	Transmission route
SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2)	COVID-19	the course of the disease is unspecific, diverse and varies greatly, from asymptomatic courses to severe pneumonia with lung failure and death	primary mode of transmission: droplet infection; smear infections and infections via the conjunctiva of the eyes are theoretically possible

The presence of pathogen or infection may be identified by

- Nucleic acid testing (NAT): e.g. RT-PCR
- Serology: detection of antibodies by e.g. ELISA

2. INTENDED USE

The GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR is intended for the qualitative determination of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) genomic RNA extracted from human respiratory (nasal wash/swab, nasopharyngeal wash/swab, oropharyngeal swab and bronchoalveolar lavage) specimen types.

3. PRINCIPLE OF THE ASSAY

The qualitative determination of specific RNA is based on Real-Time reverse-transcription Polymerase Chain Reaction (RT-PCR) technology. The kit contains specific primers and probes labelled with fluorescent reporter and quencher dyes for amplification and simultaneous detection of specific RNA sequences. Furthermore, the assay contains a heterologous amplification target (Extraction Control, EC) to identify possible RT-PCR inhibition by interfering substances contained in the sample or failure of the preceding RNA extraction. Therefore, the EC is added to the specimen during RNA isolation.

The gene of interest specific probes are labelled with the fluorophore FAM™. The Extraction Control specific probe is labelled with the fluorophore Cy5™ thereby allowing parallel detection of both amplicons in the corresponding detector channels.

4. MATERIALS PROVIDED

Cap	Symbol	Component	Volume	
green	E-MIX	RT-PCR Enzyme Mix (reaction buffer containing dATP, dCTP, dGTP, dTTP, magnesium, reverse transcriptase, RNase inhibitor protein, hot-start DNA polymerase, stabilizers, and ROX Reference Dye)	1x	750 µL
blue	PP	Primer-Probe-Mix	1x	1100 µL
yellow	EC	Extraction Control (RNA-based internal lysis, extraction and amplification control)	1x	500 µL
red	PC	Positive Control (plasmid DNA representing the N gene of SARS-CoV-2)	1x	100 µL
transparent	NFW	Nuclease Free Water	1x	500 µL

5. STABILITY AND STORAGE

The GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR kit is shipped on dry ice and all components should arrive frozen.

- All components have to be stored at -20 °C upon arrival.
- Repeated freeze thaw cycles of reagents (more than two) should be avoided, since this might affect the performance of the kit. Reagents should be frozen in aliquots if they are used intermittently.
- Keep unfrozen storage (e.g. storage on ice) as short as possible.
- Keep the E-MIX and the PP in the freezer, until you are ready to use it.
- Protect E-MIX and the PP from light.

5.1. Materials and Equipment needed, but not provided

- Real-Time PCR instrument (see 9.SPECIFIC PERFORMANCE CHARACTERISTICS)
- Equipment and consumables for isolating virus RNA from respiratory specimens
- Appropriate Real-Time PCR consumables (e.g. reaction plates, corresponding optical closing materials)
- Benchtop microcentrifuge
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Adjustable pipettes in relation to reaction setup
- Disposable DNase/RNase free pipette tips with filters
- Disposable powder-free gloves

6. SAMPLE COLLECTION AND PREPARATION

- Extracted RNA or total nucleic acid is the starting material for the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR Kit. The quality of the extracted RNA has a crucial effect on the performance of the entire RT-PCR test system. Make sure that the nucleic acid extraction method is compatible with Real-Time PCR technology.
- For nucleic acid extraction a method suitable for extracting virus RNA from human respiratory specimen should be used.
- The EC can be used for monitoring both the RNA extraction procedure and any potential PCR inhibition. Therefore, the EC has to be added prior to the nucleic acid extraction procedure. Independent of the method / system used for nucleic acid extraction, the EC can be directly added to the specimen.
- About 5 µL EC may be suitable, but should be carefully evaluated. Please refer to table under 8.1.
- Since ethanol is a strong Real-Time PCR inhibitor, it is necessary to completely eliminate it prior to the elution of the nucleic acid during extraction. If using spin columns with washing **buffers containing ethanol**, it is highly recommended to perform an additional centrifugation step of 10 min at approximately 17000 x g (~ 13000 rpm) before eluting the RNA. For this additional centrifugation step, use a new collection tube.

7. ASSAY PROCEDURE

7.1. Reaction Setup

- Please read the instructions for use carefully **before** performing the assay. Reliability of results depends on following strictly the instructions for use.
- Before use make sure that all samples and reagents are thawed completely, mixed by up and down pipetting or vortexing and centrifuged briefly.
- It is highly recommended to pipet samples and controls in triplicates.
- Pipette E-MIX slowly and carefully and use pipette tips suitable for pipetting viscous liquids.
- The use of NFW as no template control (NTC) is highly recommended.
- Define the positions of the wells on the plate for samples and controls (PC or NFW).

Reaction Setup	
E-MIX	7.5 µL
PP	10.5 µL
Sample or PC or NFW	12 µL
Total volume	**30 µL**

- Close the optical 96-well reaction plate with an optical adhesive cover.
- Centrifuge the optical reaction plate in a centrifuge with a rotor for microtiter plates for 60 seconds at approximately 1000 x g (~ 3000 rpm).

7.2. Programming the Real-Time PCR Instruments

Regarding setup and programming of the Real-Time PCR instrument, please use the manual of the respective instrument.

RT-PCR Run Settings

RT-PCR Run Settings	
Reaction Volume	30 µL
Ramp Rate	Standard
Passive Reference	ROX™

Fluorescent Detectors/Dyes

Detector	Dye	Quencher
SARS-CoV-2 RNA	FAM™	None
Extraction Control EC	Cy5™	None

Temperature Profile and Data Collection

No. of Cycles	Temperature	Time	Data Collection
1	25 °C	2 min	-
1	50 °C	30 min	-
1	95 °C	2 min	-
40	95 °C	15 sec	-
	60 °C	1 min	Fluorescence measurement at the end of every cycle

Before starting the test run, please check the settings for cycles, temperature and time.

8. RESULTS

Data analysis should be performed with the software of the used real-time PCR device according to manufacturer's instructions.

Run Validation Criteria

Test run is valid if PCR run complete
The GSD NovaPrime® SARS-CoV-2 (COVID-19) test protocol dictates that the controls be analyzed before patient sample results.
The PC, EC and NTC Ct values must meet the acceptance criteria in the table below for the assay to be valid.
If kit control(s) fail, the test is invalid and needs to be repeated.

Validation Criteria	Result/Acceptable Ct	Valid/Invalid	Measure
NTC	≥ 40	valid	-
	< 40	invalid	Repeat test run
PC	FAM™ signal no Cy5™ signal	valid	-
	no FAM™ signal	invalid	Repeat test run
EC	Cy5™ signal 28 < Ct ≤ 35	valid	-
	Cy5™ Ct > 35 and FAM™ signal ≥ 38	Sample result invalid	Repeat extraction and test run
	Cy5™ Ct ≤ 28 and FAM™ signal ≤ 38	Sample result invalid	Repeat extraction with less EC volume, repeat test run

Patient sample data is analyzed and interpreted only after all the kit controls pass.

8.1. Interpretation of Results

If test run is valid interpretation of sample results is as follows:

Detection Channel		Interpretation of Results
FAM™	**Cy5™**	
Positive Ct < 38	Positive 28 < Ct ≤ 35	The sample contains SARS-CoV-2 specific RNA.
Positive Ct < 38	Negative Ct > 35	The sample contains SARS-CoV-2 specific RNA.
Negative Ct ≥ 38	Positive 28 < Ct ≤ 35	The sample does not contain detectable amounts of SARS-CoV-2 specific RNA.
Negative Ct ≥ 38	Negative Ct > 35	PCR inhibition or reagent failure. A diagnostic statement must not be made. The RT-PCR run should be repeated or a new sample must be analysed.
Diagnosis of an infectious disease should not be established only on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as laboratory diagnostics.		

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The determinations of the specific performance characteristics were done on ABI Prism® 7500 SDS/Fast SDS (Applied Biosystems) in standard mode (7500 Software v2.3).
To establish performance characteristics, RNA extraction was performed using the bioMérieux NUCLISENS® easyMAG® or EMAG® instrument.
The results refer to the groups of samples investigated; these are not guaranteed specifications.

Use of other Real-Time PCR instruments or RNA extraction methods must be validated by the user. During validation a suitable EC volume which has to be added during RNA extraction has to be determined to obtain a valid Cy5™ signal as shown in table under 0.

For further information about the specific performance characteristics, please contact NovaTec Immundiagnostica GmbH.

9.1. Precision

Precision data for specific RNA

SARS-CoV-2	n run per day/replicates	Mean [Ct] (day1/2/3)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability				
High positive sample	1/3	15.35/15.82/15.84	0.16/0.04/0.12	**0.39/0.24/0.73**
Medium positive sample	1/3	21.47/21.70/21.79	0.16/0.43/0.11	**0.74/0.43/0.52**
Low positive sample	1/3	27.30/27.27/27.55	0.21/0.01/0.01	**0.75/0.04/0.05**
Inter-Assay Variability				
High positive sample	3/9	15.67	0.10	**0.67**
Medium positive sample	3/9	21.65	0.12	**0.56**
Low positive sample	3/9	27.37	0.08	**0.28**

9.2. Limit of Detection (LoD)

The analytical sensitivity is expressed as Limit of Detection (LoD).

	LoD [copies/reaction]
Standard cycling	3.75

9.3. Inclusivity

The analytical sensitivity of the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR is first and foremost ensured by the thorough selection of the oligonucleotides. Inclusivity was evaluated by *in silico* analysis using all publicly available SARS-CoV-2 sequences (available on the 26th of April 2020) to determine the percent identity matches for targeted sequences of the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR assay. In total 73 SARS-CoV-2 genome sequences have been analyzed by alignment to primers and probes.

9.4. Cross-Reactivity

Cross-reactivity was evaluated by *in silico* analysis against normal flora or pathogens that cause similar symptoms or pathogens related to SARS-CoV-2. Primer-probe-sets not exceeding 80 % identity to a potential cross-reacting sequence are not predicted to cause a cross-reaction.
In addition to in silico analysis, GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR was performed on nucleic acid for coronavirus 229E, coronavirus NL63, coronavirus OC43, and SARS. All pathogens tested by the GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR assay did not generate detectable amplification signals.

9.5. Negative and Positive Percent Agreement (NPA, PPA)

30 defined negative and 30 defined positive samples have been tested. 100 % agreement was achieved for all 60 samples tested.

10. QUALITY CONTROL

In accordance with NovaTec Immundiagnostica GmbH ISO-certified Quality Management System, each lot of GSD SARS-CoV-2 RT-PCR has been tested against predetermined specifications to ensure consistent product quality.

11. TRADEMARKS AND DISCLAIMERS

ABI Prism® (Applied Biosystems™), (ThermoFisher Scientific)
Registered names, trademarks, etc. used in this document are to be considered protected by law even if not specifically marked as such.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kit with other analysers than the ones mentioned under “9. SPECIFIC PERFORMANCE CHARACTERISTICS” has to be validated. Any change in design, composition and test procedure as well as any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons.
- Only for in-vitro diagnostic use.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Wear disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Always use DNase/RNase-free disposable reaction tubes and pipette tips with aerosol barriers.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- In order to avoid contamination of working space with nucleic acids, reaction tubes/plates should not be opened after amplification.
- RT-PCR is highly sensitive to nucleic acid contamination. Therefore, positive/ potentially positive material needs to be stored separate from all other components of the kit.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- This assay must not be used on the specimen directly.
- Prior to using this assay the nucleic acid has to be extracted with suitable extraction methods from the original specimen.
- Since ethanol is a strong Real-Time PCR inhibitor, it is necessary to completely eliminate it prior to the elution of the nucleic acid during extraction. If using spin columns with washing **buffers containing ethanol**, it is highly recommended to perform an additional centrifugation step of 10 min at approximately 17000 x g (~ 13000 rpm) before eluting the RNA. For this additional centrifugation step, use a new collection tube.
- The result of this RT-PCR kit may be influenced by potential mutations in the genome of the pathogen if they are located in the primer / probe binding region. Underestimation and/or failure to detect the pathogen may occur.
- PCR inhibitors may also elicit underestimation, false negative results or invalid runs. Therefore, only use nucleic acids extraction kits, which remove PCR inhibitors and which are dedicated for downstream PCR processes.
- The RT-PCR is only designed for qualified personnel who are familiar with good laboratory practice and trained in Real-Time PCR.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: PCOV6033 GSD NovaPrime® SARS-CoV-2 (COVID-19) RT-PCR (96 Determinations)

BIBLIOGRAPHY

Gorbalenya, Alexander E.; Baker, Susan C.; Baric, Ralph S.; Groot, Raoul J. de; Drosten, Christian; Gulyaeva, Anastasia A. et al. (2020): Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group (14). bioRxiv 2020.02.07.937862; doi: https://doi.org/10.1101/2020.02.07.937862

Gralinski, Lisa E.; Menachery, Vineet D. (2020): Return of the Coronavirus: 2019-nCoV. In *Viruses* 12 (2). DOI: 10.3390/v12020135.

RKI (Ed.): SARS-CoV-2 Steckbrief zur Coronavirus-Krankheit-2019 (COVID-19).
Available online at https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Steckbrief.html. (accessed: 13.04.2020)

Wang, Guan; Jin, Xian (2020): The progress of 2019 novel coronavirus event in China. In *Journal of medical virology* 92 (5), pp. 468–472. DOI: 10.1002/jmv.25705.

WHO: Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. Interim guidance. 13 March 2020.
Available online at https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected, accessed: 13.04.2020.

Zhou, Peng; Yang, Xing-Lou; Wang, Xian-Guang; Hu, Ben; Zhang, Lei; Zhang, Wei et al. (2020): A pneumonia outbreak associated with a new coronavirus of probable bat origin. In *Nature* 579 (7798), pp. 270–273. DOI: 10.1038/s41586-020-2012-7.

SYMBOLS KEY

	Manufactured by
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date
	Storage Temperature
	Protect from Light
CE	CE Mark
REF	Catalogue Number
	Consult Instructions for Use
E-MIX	RT-PCR Enzyme Mix
PP	Primer-Probe-Mix
NFW	Nuclease free water
EC	Extraction Control
PC	Positive Control
	Contains sufficient for “n” tests

NovaTec Immundiagnostica GmbH

Waldstraße 23 A6
63128 Dietzenbach, Germany

Tel.: +49 (0) 6074-48760 Fax: +49 (0) 6074-487629
Email: info@NovaTec-ID.com
Internet: www.NovaTec-ID.com

PCOV603xeng-20200504