PhoenixDx® Covid-19 VOC 4-Plex A

Covid-19 profiling kit for E484K, Deletion HV69/70, P681H & K417N

for laboratory use

qualitative RT-PCR-based profiling kit for SARS-CoV-2 variants of concern

INSTRUCTIONS FOR USE

96 Tests

REF **PCCSKU15280**

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1) Intended Use

PhoenixDx® Covid-19 VOC 4-Plex A is a real-time RT-PCR-based diagnostic test for the *in vitro* qualitative detection and discrimination of SARS-CoV-2 mutations E484K, Deletion HV69/70, K417N and P681H in respiratory specimens and sera from patients who meet COVID-19 clinical and/or epidemiological criteria.

PhoenixDx® Covid-19 VOC 4-Plex A detects SARS-CoV-2 RNA in **PhoenixDx® Gargling/Saliva Collection Kit**, nasopharyngeal and oropharyngeal swab samples during infection.

The intended use of this kit is the detection and discrimination of specific mutations in samples previously, positively tested for SARS-CoV-2. **PhoenixDx® N501Y Multiplex** is highly recommended as the first line screening kit to identify SARS-CoV-2 RNA along with the N501Y mutation.

The use of **PhoenixDx® Covid-19 VOC 4-Plex A** is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

2) PhoenixDx® Detection System

PhoenixDx® Covid-19 VOC 4-Plex A is a real-time RT-PCR-based detection and discrimination system for SARS-CoV-2. SARS-CoV-2 is considered a novel human coronavirus that is genetically distinct from the common human coronaviruses (229E, NL63, OC43, HKU1), which cause seasonal acute respiratory illness. It is also genetically distinct from the two newer human coronaviruses, MERS-CoV and SARS-CoV. Since end of 2020, novel lineages occurred in its genome which alter the viral performance of binding the human ACE receptor, increasing its mortality or evasion of immune response.

PhoenixDx® Covid-19 VOC 4-Plex A detects 4 different mutations in S gene of SARS-CoV-2 in a multiplex approach to identify a specific lineage like B.1.1.7 (UK variant). Additionally, two non-infectious target positive controls (**TPC Wildtype and Mutant**) are included. The positive control is used to confirm functionality of the assays and overall PCR performance.

2.1) qPCR-based Detection

The first step in the detection and discrimination of SARS-CoV-2 mutations is the conversion of viral RNA into cDNA. Afterwards, the target sequences are simultaneously amplified in one reaction with amplification monitored in real time through the use of fluorescently labelled probes: upon incorporation into the newly amplified DNA strands, the fluorophore is released and an increase in fluorescence signal can be observed.

Due to the intrinsic mutation rate of coronaviruses, it is possible that mutations in the target sequence occur and accumulate over time. This can lead to false-negative results with a PCR-based detection approach.

PHOENIXDX® COVID-19 VOC 4-PLEX A is compatible with every qPCR cycler with calibrated Cy5, ROX, HEX/VIC and FAM™.

2.2) MATERIALS PROVIDED

QUANTITY AND VOLUME	COMPONENT
1x 100 µl	20X RT Enzyme Mix
1x 400 µl	5X MTM Buffer
1x 200 µl	10X VOC 4-Plex Assay Mix
1x 100 µl	TPC SC2-WT
1x 100 µl	TPC SC2-Mutant

2.3) ADDITIONAL MATERIALS REQUIRED

- Suitable means & equipment for nucleic acid extraction (see chapter 3.4)
- Real-time PCR detection system equipped for Cy5, ROX, HEC/VIC and FAM™ detection
- Adjustable pipettes & fitting filtered pipette tips
- Nuclease-free water
- Appropriate PSA & workspaces for working with potentially infectious samples
- Surface decontaminants such as DNAZap™ (Life Technologies), DNA Away™ (Fisher Scientific), RNAse Away™ (Fisher Scientific), 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)
- Nuclease-free tubes / strips / plates to prepare dilutions, mastermixes etc.
- Nuclease-free tubes / strips / plates corresponding to the PCR device
- Suitable storage options for reagents and specimen (4°C, -20°C, -70°C)

2.4) STORAGE

- Store all components at -20°C and avoid repeated freeze and thaw cycles (≤ 3 freeze/thaw cycles; prepare aliquotes if required).
- Protect the **10X VOC 4-Plex Assay Mix** from light as prolonged exposure can diminish the performance of the fluorophores.
- If the kit components have been damaged during transport, contact Procomcure Biotech. Do not use as performance may be compromised.
- Keep reagents separate from sample material to avoid contamination.
- Do not use after the designated expiry date.

3) CONSIDERATIONS BEFORE STARTING

3.1) BIOSAFETY

- Wear appropriate personal protective equipment (e.g. gowns, powder-free gloves, eye protection) when working with clinical specimens.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.

- For more information, refer to:
  - Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-COV-2) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
  - Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.
- The use of **PHOENIXDX® COVID-19 VOC 4-PLEX A** and data evaluation is restricted to trained laboratory personnel only.
- Good laboratory practice is essential for optimal performance of this assay. Special care must be taken avoid contamination of the components of the kit. All reagents must be closely monitored for impurities and contamination. Discard suspicious reagents according to local guidelines and regulations.

3.2) SPECIMENS

Only use appropriate specimens for testing, such as:

- Respiratory specimens including nasopharyngeal / oropharyngeal aspirates or washes, nasopharyngeal / oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates and sputum.
- Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not recommended as they may contain substances that inactivate some viruses and inhibit PCR testing and should only be used if dacron or rayon swabs are not available.
- **PhoenixDx® Gargling/Saliva Collection Kit** is a recommended choice of sample collection.

3.3) SPECIMENS - HANDLING AND STORAGE

- Specimens can be stored at 4°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Clinical specimens must be considered potentially infectious and treated accordingly.

⚠ Do not vortex specimens as this will fragment the RNA and lead to failure of **PHOENIXDX® COVID-19 VOC 4-PLEX A.**

Do not use specimens if

- they were not kept at 2-4°C (≤ 4 days) or frozen at -70°C or below.
- they are insufficiently labelled or lack documentation.
- they are not suitable for this purpose (see above for suitable sample material).
- the specimen volume is insufficient.

3.4) Sample Preparation / Nucleic Acid Extraction

- The performance of RT-PCR assays strongly depends on the amount and quality of sample template RNA. It is strongly recommended to qualify and validate RNA extraction procedures for recovery and purity before testing specimens.
- Suitable nucleic acid extraction systems successfully used in combination with **PhoenixDx® Detection Kits** include: SphareraMag® DNA/RNA Isolation Kit (Procomcure Biotech), Quick-RNA Viral Kits (Zymo Research), bioMérieux NucliSens® systems, QIAamp® Viral RNA Mini Kit, QIAamp® MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche MagNA Pure Compact RNA Isolation Kit, Roche MagNA Pure Compact Nucleic Acid Isolation Kit.
- Only extract the number of specimens that will be tested in a single day.
- Do not freeze/thaw extracts more than once before testing as each freeze/thaw cycle will decrease the RNA quality. For optimal results, use directly and do not freeze and thaw before use.
- Extracted nucleic acids should be stored at -70°C or lower and (if re-testing is expected) stored in aliquots.

3.5) Reaction Setup

1) Make sure that all necessary equipment and devices are suitable, calibrated and functional before starting the experiments.
2) Decontaminate equipment and workspace and prepare everything needed for the following experiment to keep the workflow short and repeatable.
3) Switch on the PCR detection system and program it to avoid delays after setting up the reactions.
4) Thaw all components of **PhoenixDx® Covid-19 VOC 4-Plex A** on ice and mix gently but thoroughly to ensure even distribution of components. Collect liquid at the bottom of the tube with a quick spin.
5) Set up your **Mastermix Plate**:

    a. Always prepare control reactions with nuclease-free dH_2O instead of sample material **(NTC)** to detect contamination in your reagents.
    b. When using the provided target positive controls **(TPC)**, use **13 µl / reaction**.
    c. > 2 replicates / sample are strongly recommended.
    d. Prepare enough mastermix for all planned reactions. It is recommended to prepare mastermix for 2 additional reactions to compensate for pipetting inaccuracies.
    e. Distribute the mastermix to your strips/plate.

Component	Volume
20X RT Enzyme Mix	1 µl
5X MTM Buffer	4 µl
10X VOC 4-Plex Assay Mix	2 µl
Isolated sample RNA / TPC / NTC	13 µl / 13 µl / 13µl dH_2O

6) Transfer the Mastermix Plate to a separate workspace to add the sample material. Preparing reagents and final reaction setup in separate workspaces helps to avoid contamination of equipment and reagents with sample material.

   a. Prepare negative reactions first and seal them before handling positive samples. It is recommended to only bring potentially positive sample material and the included target positive control to the workspace once the NTC is prepared and sealed.
   b. Add your samples to the Mastermix Plate.
   c. Keep reactions on ice until transferring them to the PCR device.

7) Transfer the reactions to the PCR device, then cycle according to these guidelines:

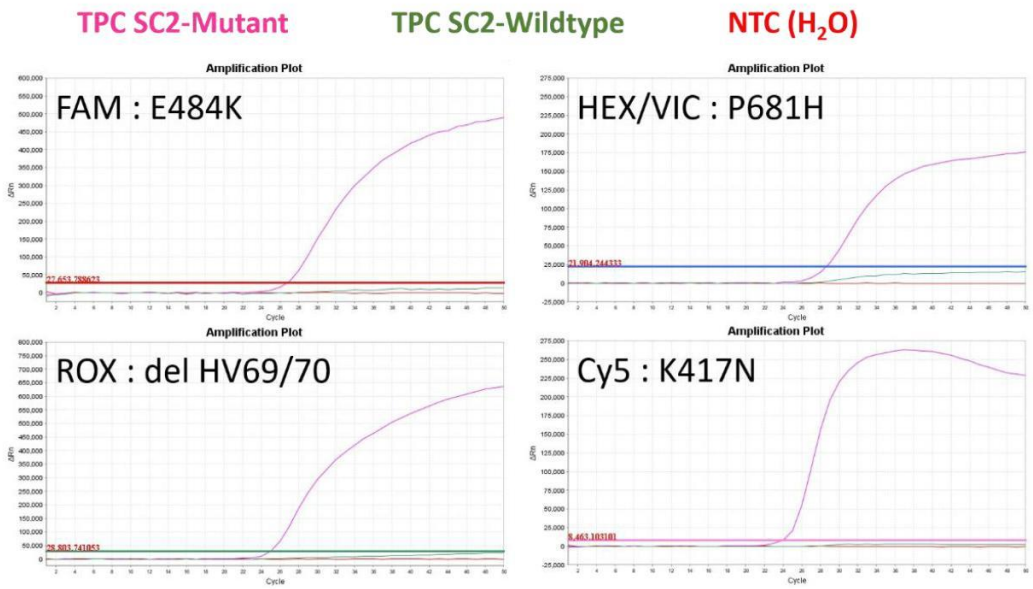
Step	Cycles	Temperature	Duration
Reverse Transcription	1	50°C	5 minutes
Initial Denaturation	1	95°C	5 minutes
Amplification	50	95°C	5 seconds
		59°C[1]	45 seconds

[1] Enable Data Collection for **FAM™** (E484K), **ROX** (Deletion HV69/70), **HEX/VIC** (P681H) and **Cy5** (K417N). Do not set **ROX** as passive reference since the channel is used for detection.

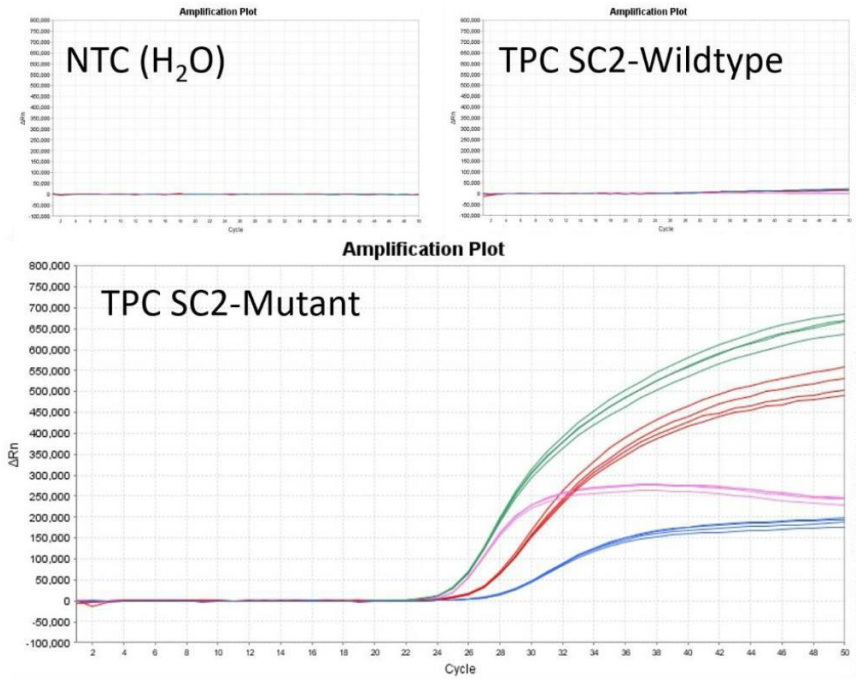
Once the run is finished, do not open the reaction tubes to avoid contamination and discard according to local guidelines and regulations. Do not autoclave as this may contaminate laboratory equipment with amplicons.

4) Analysis

Adjust threshold of every detection channel above NTC and TPC SC2-Wildtype fluorescence as shown below:



- **dH_2O controls (NTC) and TPC SC2-Wildtype must not give a Ct value for any target**. If they do, the reaction was contaminated with sample RNA / cDNA. Decontaminate equipment and workspace and repeat the reactions. Also, check for device-derived artifacts or falsely placed threshold. **If a contamination persists, use fresh reagents**.
- **TPC SC2-Mutant must give a Ct value for all four targets below Ct 35.**



Always analyze your sample reactions independently of the TPC reactions. The TPC is an artificial control construct resulting in a significantly higher signal strength than actual samples. This will lead to a distorted picture when analyzed together with actual samples.

FAM™	HEX/VIC	ROX	Cy5	Interpretation
+	+	+	+	**All channels give a positive CT value. Expected result for TPC SC2-Mutant.**
≥ 1 channel gives positive Ct value				**Check Lineage Chart for detailed profile interpretation.**
/	/	/	/	**No channel must give a positive CT value after threshold adjustment. Expected result for TPC SC2-Wildtype.**
/	/	/	/	**No channel must give a positive CT value after threshold adjustment. Expected result for no template control (NTC).**

Table 1 Interpretation of amplification results with PhoenixDx® Covid-19 VOC 4-Plex A.

	PDx N501Y Multiplex		PDx Covid-19 VOC 4-Plex A			
SNPs	**SARS-CoV-2**	**N501Y**	**K417N**	**del HV69/70**	**E484K**	**P681H**
DK (Mink Cluster V)	X			X		
UK B.1.1.7	X	X		X		X
UK B.1.1.7 (Bristol Variant)	X	X		X	X	
ZA B.1.351 (N501Y.2)	X	X	X		X	
BRA B.1.1.28 (P.1)	X	X			X	
BRA B.1.1.28 (P.2)	X				X	
BRA B.1.1.28 (P.3)	X	X			X	X
B.1.1.207 (Nigeria)	X					X
B.1.258	X			X		
B.1.525 (global, also UK)	X			X	X	
B.1.526-E484K (New York)	X				X	

Table 2 Lineage chart for profiling with PhoenixDx® N501Y Multiplex as first line screening kit and PhoenixDx® Covid-19 VOC 4-Plex A in combination.

5) Limitations

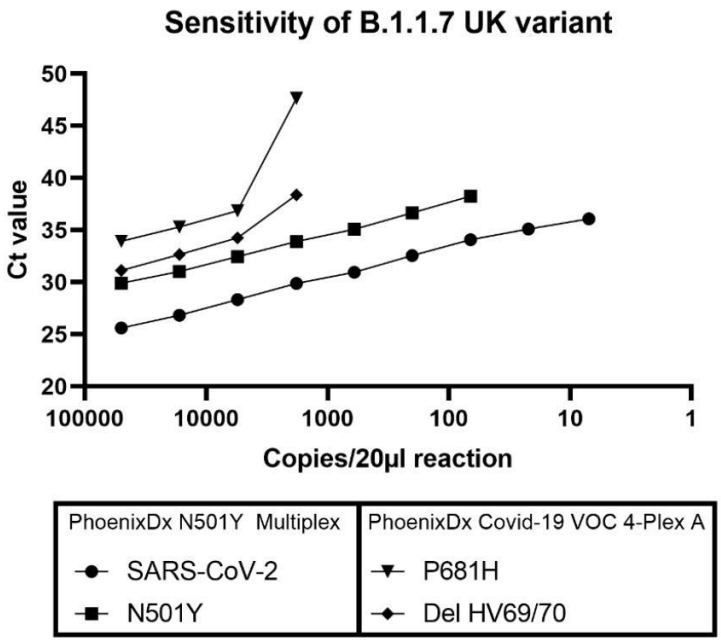
- For reliable results, it is essential to adhere to the guidelines given in this manual. Changes in reaction setup or cycling protocol may lead to failed experiments.
- Depending on the sample matrix, inhibitors may be present in the isolated RNA and disable reverse transcription and / or PCR amplification. If this is the case, another sample type or isolation method may be beneficial.
- Spontaneous mutations within the target sequence may result in failure to detect the target sequence.
- **Any signal recorded in the detection channels may only be analyzed if the corresponding SARS-CoV-2 Ct value in the previous run was ≤ 30.**
- For safety reasons, specimen collection, transport, storage and processing procedures must be performed by trained personnel only.
- This assay must not be used on specimens directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- Reliable results depend strongly on proper sample collection, storage, RNA isolation and handling procedures.

6) Quality Control

In accordance with Procomcure Biotech GmbH's EN ISO 13485-certified Quality Management System, each lot of **PhoenixDx® Covid-19 VOC 4-Plex A** is tested against predetermined specifications to ensure consistent product quality.

7) Analytical Sensitivity

The LOD95(Limit of Detection) defines the number of target sequences (copy number) that can be detected in ≥ 95% of reactions. The LOD95 was determined by testing a serial dilution of isolated SARS-CoV-2 RNA of the B.1.1.7 variant with 9 concentrations in 4 replicates per concentration. In parallel the PhoenixDx® N501Y Multiplex was used to show why the threshold of ≤ 30 of a previous SARS-CoV-2 detection was set as a limitation for a subsequent profiling approach.



The **PhoenixDx® Covid-19 VOC 4-Plex A** reliably detected the P681H mutation and the Deletion HV69/90. Ct values for K417N and E484K were undetermined. In addition to the data of the first line PCR with **PhoenixDx® N501Y Multiplex,** the specific lineage of B.1.1.7 (first UK variant) can be reported. The sensitivity of the **PhoenixDx® Covid-19 VOC 4-Plex A** corresponds to ~140 copies of viral RNA per microliter in the purified sample.

8) Trademarks

PhoenixDx®, NucliSens® (bioMérieux), QIAamp®, RNeasy® (QIAGEN), ChargeSwitch® (Invitrogen), ROX™, FAM™ (Life Technologies), DNAZap™, DNA Away™, RNAse Away™

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9) Literature

Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045

10) Compatible Cycler

Applied Biosystems 7500, Applied Biosystems 7500FAST, Applied Biosystems QuantStudio 5, Applied Biosystems Viia7, Biorad CFX.

[for all listed cyclers with 96-well block and valid calibration for FAM, HEX/VIC, ROX and Cy5]

11) Technical Assistance

For questions or technical support, contact Procomcure Biotech:

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12) Symbol Definition (Manual & Packaging)

	Contains sufficient for <n> tests
	Catalogue Number
	Manufacturer
	Batch Code
	Temperature Limit
	Use-by Date
	Consult instructions for use