Primerdesign™ Ltd

Primerdesign Ltd

PROmate™ COVID-19

96 reactions

Total workflow solution for the qualitative detection of SARS-CoV-2 viral RNA. For use with anterior nasal and oropharyngeal specimens collected as dry swabs

NOT FOR USE WITH SWABS STORED IN GUANIDINIUM THIOCYANATE-CONTAINING MEDIA

Instructions for Use (IFU) *Issue 10.00*

PROmate COVID-19
D00050, D00051, D00068, D00070
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Primerdesign Ltd

PROmate™ COVID-19

In vitro Real-Time PCR diagnostic test for Coronavirus COVID-19

For Use with:

Sample Types	Extraction Platforms	PCR Platform
Anterior Nasal swab and oropharyngeal swab	PROmate™	genesig® q16/q32 (Primerdesign, Novacyt)

96 tests

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D00050, D00051, D00068, D00070

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1. Intended Use

PROmate™ COVID-19 is a total workflow solution, inclusive of sample preparation, qPCR amplification and analysis on the genesig® q16 and q32 instruments, specifically for the detection of SARS-CoV-2.

PROmate™ COVID-19 is intended for the qualitative detection of nucleic acid from SARS-CoV-2 from anterior nasal and oropharyngeal specimens prepared on dry swabs. The assay provides rapid screening of individuals for SARS-CoV-2 infection and aids the diagnosis of suspected COVID-19 in patients. The assay is intended for use with the designated genesig® q16 and q32 qPCR platforms.

PROmate™ COVID-19 is intended for use by trained personnel specifically instructed and trained in handling SARS-CoV-2 positive samples and the techniques of real-time PCR and in vitro diagnostic procedures.

2. Summary and Explanation

An outbreak of pneumonia of unknown aetiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) in December 2019. Chinese authorities identified a novel coronavirus SARS-CoV-2 (previously called 2019-nCoV) which has resulted in confirmed human infections worldwide and cases of COVID-19 disease. Symptoms of COVID-19 disease include severe respiratory illness and has resulted in the death of patients. Patients can become infected with SARS-CoV-2 virus by person-person contact (through contact with a contaminated environment or person).

PROmate™ COVID-19 workflow is a combination of a direct to PCR sample processing method and molecular *in vitro* diagnostic test for the detection of the SARS-CoV-2 RNA from anterior nasal and oropharyngeal dry swabs for interpretation on the genesig® q16 and q32. The viral RNA is released from the swab sample during incubation with a viral inactivation / lysis agent. Following the swab sample preparation process, an aliquot of the resulting sample is tested using well-established nucleic acid amplification technology in the genesig® Coronavirus (COVID-19) assay. The PCR assay contains oligonucleotide primers and dual-labeled hydrolysis probes, as well as control material, for use in Real-Time RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA.

3. Principles of the Procedure

Viral RNA is released from anterior nasal or oropharyngeal dry swabs using 'direct to PCR' technology. Using polymerase chain reaction (PCR) technology, the RNA is reverse transcribed to cDNA and subsequently amplified using forward and reverse primers. A fluorescent labelled probe is used to detect the amplicon. The probe system is based on the standard hydrolysis probe system known as TaqMan® Technology and the probes are labelled with fluorescent reporter and quencher dyes.

During PCR cycling, the probe anneals to a specific target sequence located between the forward and reverse primers. The probe is cleaved by the 5' nuclease activity of the Taq polymerase during the extension phase of the PCR cycle, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each PCR cycle, additional reporter dye molecules are released from the probe, increasing the fluorescence intensity. Fluorescence intensity is recorded at each cycle of the PCR by the Real-Time PCR machine.

PROmate™ COVID-19 includes SARS-CoV-2 specific primers and a SARS-CoV-2 specific probe labelled with the FAM fluorophore. The primer/probe mix also includes primers and probes to amplify and detect an Internal Control (IC) RNA template. This is simultaneously amplified along with any target SARS-CoV-2 RNA and provides a process control for identification of a successful PCR event. The IC specific probe is labelled with the HEX fluorophore.

The genesig® IC RNA template is not related to the SARS-CoV-2 viral sequence. The purpose of the IC is to monitor the integrity of the PCR run and control for inhibition from patient samples, however it does not confirm the presence of human material.

The oligonucleotide primers and probe for the detection of SARS-CoV-2 were selected from the ORF1ab genomic region. The supplied primer/probe mix is designed for the specific detection of SARS-CoV-2 RNA only.

PCR amplification using the included chemistry and plasticware in PROmate™ COVID-19 is designed specifically for use on genesig® q16 and genesig® q32 instruments. The reagents are provided pre-loaded in magazines which will slot into the instruments to facilitate ease of use. For users operating PROmate™ COVID-19 where turnaround requires incomplete runs, separate controls and magazines are available for total flexibility with sample numbers.

4. Materials Provided

PROmate™ COVID-19 is presented in a format which is suitable for running 16 reactions over 6 separate runs (genesig® q16 users) or 3 separate runs (genesig® q32 users). PROmate™ COVID-19 is prepared in 2 separate pack types for the ease of transportation and storage. For users who wish to operate partial runs with PROmate™ COVID-19, accessory packs should be used to provide additional controls and magazines (q16 pack catalogue: A00100, q32 pack catalogue: A00101).

genesig® q16 (Catalogue: D00050)

The reagents required for each run come in two different packs; an ambient box and a frozen pouch received on dry ice. The PROmate™ box contains 12 bags (6 foils and 6 zip-lock bags) and should be stored at ambient temperature. Reagents included in the PROmate™ box are summarised in Table 1 below.

Table 1: Pack 1: PROmate™ COVID-19 Box
(6x foil pouches and 6x clear plastic zip-lock bags)

Reagent label	Number of vials (16 tests)	Volume (µl per vial)	Lid colour
PROmate™ COVID-19 RNase inhibitor	14	n/a	n/a
PROmate™ COVID-19 Sample Preparation Buffer	14	1000	n/a
PROmate™ COVID-19 Negative Control Solution	1	50	Red

The PROmate™ pouch comes on dry ice and should be stored frozen (-25°C to -15°C). Upon opening, the pack contains 6 PROmate™ COVID-19 Positive Controls and 6 PROmate™ COVID-19 16 reaction magazines as below in Table 2.

Table 2: Pack 2: PROmate™ COVID-19 Foil Pouch
(6x foil pouches with positive control and 6x foil pouches with magazine and *1x spares pack of Mastermix tubes provided*)

Reagent label	Number of pieces (16 tests)	Volume (µl per vial)	Lid/foil colour
PROmate™ COVID-19 Positive Control	1	20	Gold Translucent Foil
PROmate™ COVID-19 Mastermix magazine	1	15	Silver Foil
PCR tube lids	15	n/a	Clear
PROmate™ COVID-19 Spare Mastermix	3	15	Clear

genesig® q16 (Catalogues: D00068)

The reagents required for each run come in two different packs; an ambient box and a frozen pouch received on dry ice. The PROmate™ box contains 12 bags (6 foils and 6 zip-lock bags) and should be stored at ambient temperature. Reagents included in the PROmate™ box are summarised in Table 3 below.

Table 3: Pack 1: PROmate™ COVID-19 Box
(6x foil pouches and 6x clear plastic zip-lock bags)

Reagent label	Number of vials (16 tests)	Volume (µl per vial)	Lid colour
PROmate™ COVID-19 RNase inhibitor	14	n/a	n/a
PROmate™ COVID-19 Sample Preparation Buffer	14	1000	n/a
Sample Labels	14	n/a	n/a

The PROmate™ pouch comes on dry ice and should be stored frozen (-25°C to -15°C). Upon opening, the pack contains 6 PROmate™ COVID-19 Positive Controls, 6 PROmate™ COVID-19 16 reaction magazines and 6 negative control solutions as below in Table 4.

Table 4: Pack 2: PROmate™ COVID-19 Foil Pouch
(6x foil pouches with positive control, 6x foil pouches with magazine, 1x bag negative control solution and *1x spares pack of Mastermix tubes provided*)

Reagent label	Number of pieces (16 tests)	Volume (µl per vial)	Lid/foil colour
PROmate™ COVID-19 Positive Control	1	20	Gold Translucent Foil
PROmate™ COVID-19 Mastermix magazine	1	15	Silver Foil
PCR tube lids	15	n/a	Clear
PROmate™ COVID-19 Negative Control Solution	1	50	Red
PROmateTM COVID-19 Spare Mastermix	3	15	Clear

genesig® q32 (Catalogue: D00051)

The reagents required for each run are separated within each of the two pack types. The PROmate™ box contains 6 bags (3 foils and 3 zip-lock bags) and should be stored at ambient temperature. This is shown below in Table 5.

Table 5: Pack 1: PROmate™ COVID-19 Box
(3x foil pouches and 3x clear plastic zip-lock bags)

Reagent label	Number of vials (32 tests)	Volume (µl per vial)	Lid colour
PROmate™ COVID-19 RNase inhibitor	30	n/a	n/a
PROmate™ COVID-19 Sample Preparation Buffer	30	1000	n/a
PROmate™ COVID-19 Negative Control Solution	1	50	Red

Pack 2 comes on dry ice and should be stored frozen (-25°C to -15°C). Upon opening, the pack contains 3 PROmate™ COVID-19 Positive Controls and 3 PROmate™ COVID-19 32 reaction magazines as below in Table 6.

Table 6: Pack 2: PROmate™ COVID-19 Pouch
(3x foil pouches with positive control, 3x foil pouches with magazine and *1x spares pack of Mastermix tubes provided*)

Reagent label	Number of pieces (32 tests)	Volume (µl per vial)	Lid/foil colour
PROmate™ COVID-19 Positive Control	1	20	Gold translucent Foil
PROmate™ COVID-19 Mastermix magazine	1	15	Silver Foil
PCR tube lids	31	n/a	Clear
PROmate™ COVID-19 Spare Mastermix	3	15	Clear

genesig® q32 (Catalogues: D00070)

The reagents required for each run are separated within each of the two pack types. The PROmate™ box contains 6 bags (3 foils and 3 zip-lock bags) and should be stored at ambient temperature. This is shown below in Table 7.

Table 7: Pack 1: PROmate™ COVID-19 Box

(3x foil pouches and 3x clear plastic zip-lock bags)

Reagent label	Number of vials (32 tests)	Volume (µl per vial)	Lid colour
PROmate™ COVID-19 RNase inhibitor	30	n/a	n/a
PROmate™ COVID-19 Sample Preparation Buffer	30	1000	n/a
Sample Labels	30	n/a	n/a

Pack 2 comes on dry ice and should be stored frozen (-25°C to -15°C). Upon opening, the pack contains 3 PROmate™ COVID-19 Positive Controls, 3 PROmate™ COVID-19 32 reaction magazines and 3 negative control solutions as below in Table 8.

Table 8: Pack 2: PROmate™ COVID-19 Pouch

(3x foil pouches with positive control, 3x foil pouches with magazine, 1 x bag negative control solution and *1x spares pack of Mastermix tubes provided*)

Reagent label	Number of pieces (32 tests)	Volume (µl per vial)	Lid/foil colour
PROmate™ COVID-19 Positive Control	1	20	Gold translucent Foil
PROmate™ COVID-19 Mastermix magazine	1	15	Silver Foil
PCR tube lids	31	n/a	Clear
PROmate™ COVID-19 Negative Control Solution	1	50	Red
PROmate™ COVID-19 Spare Mastermix	3	15	Clear

genesig® q16 (Catalogue: A00100)

The reagents required for each run come in a frozen pouch received on dry ice and should be stored frozen (-25˚C to -15˚C). Upon opening, the pack contains 8 PROmate™ COVID-19 q16 magazines with negative control solution mastermix and 8 PROmate™ COVID-19 Positive Controls as below in Table 9.

Table 9: PROmate™ COVID-19 Control Pack
(8x foil pouches with positive control and 8x foil pouches with magazine)

Reagent label	Number of pieces (16 tests)	Volume (µl per vial)	Lid/foil colour
PROmate™ COVID-19 Positive Control	1	20	Gold translucent Foil
PROmate™ COVID-19 q16 magazine with negative control solution mastermix	1	15	Silver Foil
PCR tube lids	15	n/a	Clear

genesig® q32 (Catalogue: A00101)

The reagents required for each run come in a frozen pouch received on dry ice and should be stored frozen (-25˚C to -15˚C). Upon opening, the pack contains 8 PROmate™ COVID-19 q32 magazines with negative control solution mastermix and 8 PROmate™ COVID-19 Positive Controls as below in Table 10.

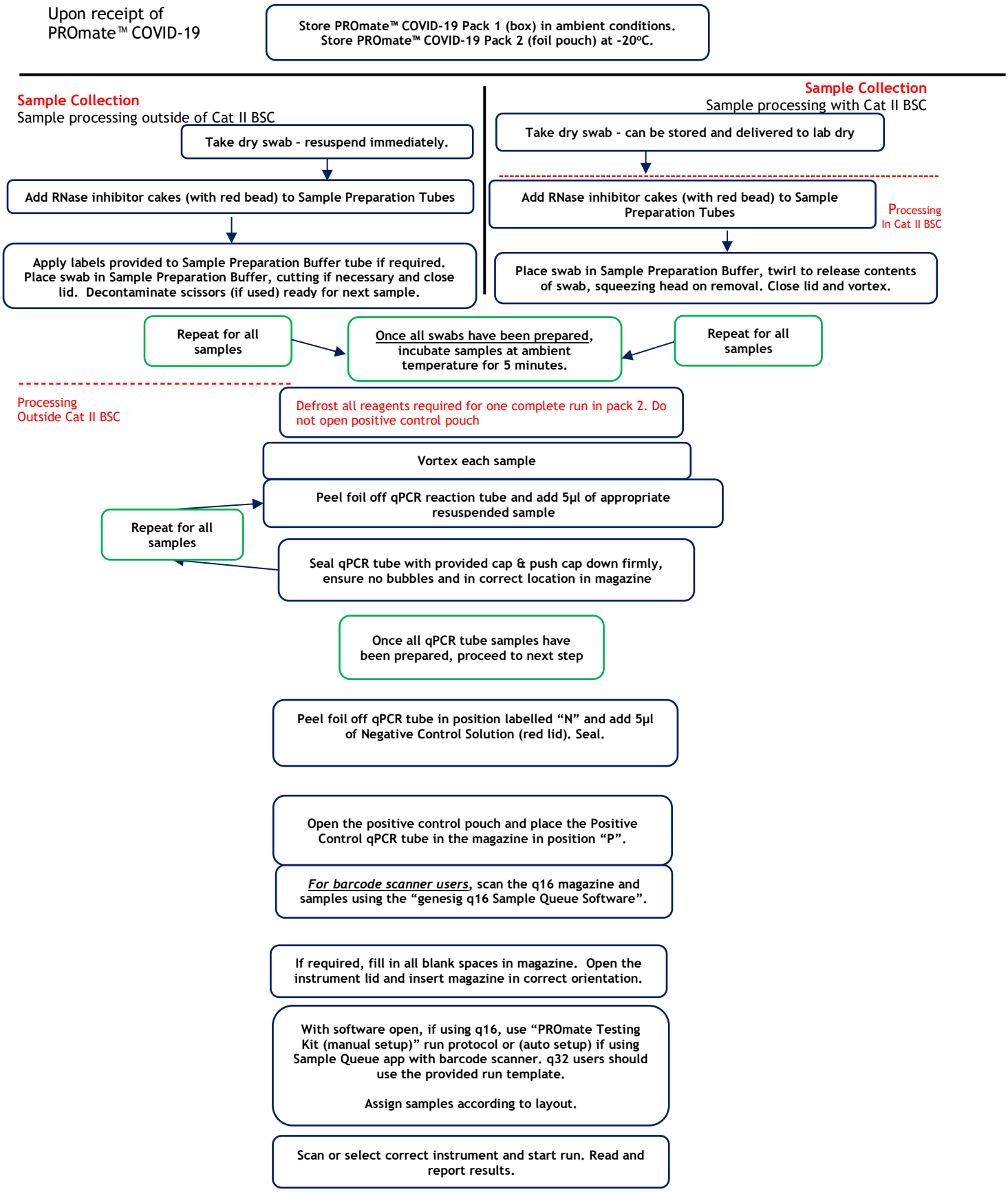
Table 10: PROmate™ COVID-19 Control Pack
(8x foil pouches with positive control and 8x foil pouches with magazine)

Reagent label	Number of pieces (32 tests)	Volume (µl per vial)	Lid/foil colour
PROmate™ COVID-19 Positive Control	1	20	Gold translucent Foil
PROmate™ COVID-19 q32 magazine with negative control solution mastermix	1	15	Silver Foil
PCR tube lids	31	n/a	Clear

The PROmate™ COVID-19 primer/ probe mix contains the primers and FAM labeled probe specific to SARS-CoV-2 and includes the primers and HEX/VIC labeled probe specific to the genesig® RNA IEC.

The PCR Mastermix, PROmate™ COVID-19 primer/ probe, IEC primer/ probe and template are provided together, ready within the reaction tubes in the Mastermix magazine. The PROmate™ COVID-19 Positive Control is provided ready prepared in its own reaction tube and foil pouch to avoid contamination.

5. Summary of Preparation and Testing Process



6. Required Equipment and Consumables (Not Provided)

- Vortex
- Microcentrifuge (CL2 users) (Optional)
- Adjustable 10µl or 20µl micropipette, or a fixed 5µl micropipette
- Aerosol barrier pipette tips with filters
- 0.1ml qPCR tubes (lid balancing on partial runs)
- Disposable gloves
- Scissors (optional)
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- 70% Alcohol (either ethanol or isopropanol)
- Barcode scanner (Optional)

7. Real-Time PCR instruments

The PROmate™ COVID-19 assay has been validated with the following Real-Time PCR instruments:

- genesig® q16 (Primerdesign, Novacyt Group, software version 2.10.5)
- genesig® q32 (Primerdesign, Novacyt Group, software version 1.2.2)

N.B. *please ensure that all instruments used have been installed and maintained according to the manufacturer's instruction and recommendations.*

8. Facilities/Training Requirements

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures:

Refer to the UK Government guidance on handling and processing potential COVID-19 samples in laboratories:

www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens

Refer to the World Health Organization Interim guidance on laboratory biosafety: www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance from 13 May 2020.

Refer to the Centers for Disease Control and Prevention (CDC) guidelines:

Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

9. Warnings and Precautions

9.2 9.1 General

- Handle all specimens as if infectious using safe laboratory procedures. Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) microbiological safety cabinet (refer to the guidance detailed in **Section 8**).
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited) gloves and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes and other equipment and reagents.
- Please consult the safety data sheet (SDS) before using this kit, which is available on request.
    - The PROmate™ Sample Preparation Buffer contains EGTA. This component should be handled according to the SDS. In the event of damage to protective packaging, contact Primerdesign for instructions.
    - The PROmate™ Sample Preparation Buffer contains Triton X-100 Reduced. This component should be handled according to the SDS. This product is hazardous to the environment and should be disposed of as detailed in the SDS. In the event of damage to protective packaging, contact Primerdesign for instructions.

9.3 9.2 Preventing Contamination

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).
- The PROmate™ COVID-19 Positive Control is provided in a sealed pouch and contains a high copy number template. **Before** opening the positive control pouch, visually inspect for leakage. If there is evidence of leakage inside the bag, **DO NOT OPEN** and use a replacement positive control to avoid contamination. The sealed lid on the PCR reaction tube for the positive control should not be opened to avoid cross-contamination.
    - **DO NOT** open reaction tubes once PCR has been completed.
    - **Never** open the positive control reaction tube, before or after PCR
    - Maintain separated, dedicated equipment (e.g., pipettes, microcentrifuge) and supplies (e.g., microcentrifuge tubes, pipette tips) for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
    - Wear a clean lab coat and disposable gloves when setting up assays.
    - Change gloves regularly and whenever contamination is suspected.
    - Keep reagent and reaction tubes capped or covered as much as possible.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimise the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the sample preparation procedure. Good aseptic technique should always be used when working with nucleic acids.
    - Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g.,10% bleach) to minimise risk of nucleic acid contamination.
- RNA samples should be maintained on a cold block or on ice during preparation to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with 10% bleach.
- Handle post-amplification PCR tubes with care to ensure that the seal is not broken.
- Dispose of human biological samples according to national and international regulations (refer to guidance detailed in **Section 8**).

9.4 9.3 Prevent DNase/RNase contamination

- Use DNase/RNase free disposable plasticware and pipettes reserved for DNA/RNA work to prevent cross-contamination with DNases/RNases from shared equipment.
- Use DNase/RNase free filter tips throughout procedure to prevent aerosol and liquid contamination.

10. Reagent Storage, Handling and Stability Conditions

10.2 10.1 Storage conditions

- The PROmate™ COVID-19 Pack 1 is shipped at ambient temperatures. This can be stored at ambient upon arrival.
- The PROmate™ COVID-19 Pack 2 is shipped frozen, on dry ice. Upon arrival the contents should be stored at -20°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Once the "use by" date has been reached, the kit components should be discarded following the disposal instructions in **Section 19.**
- If the kit's protective packaging is damaged upon receipt, please contact Primerdesign for instructions.

10.3 10.2 In-use Stability

PROmate™ COVID-19 is pre-dispensed, with the operator selecting the number of reactions and controls required as appropriate to complete their run. As such, there is no necessity to make bulk reagents and dispense, facilitating ease of use for the operator. Reagents should be stored at their specified temperatures and only removed from storage immediately prior to use only.

If operating partial runs, the necessary number of master mix reaction tubes will need to be removed and saved for subsequent runs. In this scenario, the magazine should be removed from frozen storage, the desired number of reaction tubes removed from the magazine (to be used on a later run) and then immediately replaced in the provided storage conditions. Defrosting should not be allowed to occur to avoid freeze-thaw cycling.

11. Specimen Collection, Handling and Storage

11.1 Compatible Specimens

- This product is intended for use with dry swabs only.
- This product is not intended for use with swab material stored in viral transport media, guanidinium thiocyanate-containing media or any other liquid collection solution. Use of media not supplied as part of this product will impact the limit of detection of the device.
- **Samples that present with obvious blood or other particulate matter are NOT compatible with PROmate™ COVID-19 and should be discarded**

11.2 Collecting the Specimen Compatible Specimens

Sampling should be conducted with the correct swab type and collected following the correct sampling technique. CDC guidance on collection of anterior nasal swabs can be found here: https://www.cdc.gov/coronavirus/2019-ncov/downloads/community/COVID-19-anterior-self-swab-testing-center.pdf

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13 (Clinical and Laboratory Standards Institute) may be referenced as an appropriate resource.

	Anterior Nasal swabs
Collection	Swabs: Dry dacron or polyester/nylon flocked swabs in a sterile container
Transport temperature	2-8ºC ≤ 72hrs (dry swab)* <4ºC ≤ 24hrs (resuspended swab) 4-20ºC ≤ 4hrs (resuspended swab)
Long-term storage	≤ -70˚C for longer periods (dry swab)*

*These are CDC recommendations: CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html, Local regulations pertaining to sample handling may also apply.

- Swab specimens should be collected and placed in a clean, dry, sterile transport prior to testing.
- For dry swabs for use with Cat II BSC, swab specimens must be transported within 24 hours and tested as soon as possible after collection. If this is not possible, the following storage recommendations should be followed:
    - Swab samples must be transported within 24 hours or stored refrigerated.
    - If the swab is stored at 2-8˚C, the specimen must be tested within 72 hours.
    - If testing cannot be conducted within 72 hours, the swab specimen should be frozen at -70˚C or colder until testing is able to be conducted.
- For resuspended samples for use outside Cat II BSC, swab specimens must be transported within 4 hours and tested as soon as possible after collection. If this is not possible, the following storage recommendations should be followed:
    - Swab samples must be transported within 4 hours or stored refrigerated.
    - If the swab is stored at <4˚C, the specimen must be tested within 24 hours.

- For further specimen guidance please refer to the following:
    - UK Government guidance on handling and processing potential COVID-19 samples in laboratories: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens
    - World Health Organization Interim guidance on laboratory biosafety from 13 May 2020: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance
- Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- The PROmate™ Sample Preparation Buffer contains Triton X-100 Reduced (Triton X-100 replacement) and has been utilised for the inactivation of SARS-CoV-2 by Public Health England. Therefore, samples should be handled according to these revised national guidelines for sample management prior to inactivation.
- Follow specimen collection devices manufacturer instructions for proper collection methods.
- Swab specimens should be collected using swabs with a synthetic tip, such as nylon or Dracon® and with an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.

11.3 Transporting Specimens

- Specimens must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

12. General Preparation

12.1 Equipment Preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use.
- Decontamination agents should be used such as 10% bleach to minimise the risk of nucleic acid contamination.

13. Assay Set Up

13.1 Procedure Caution

The following advice should be adhered to in order ensure consistent, accurate results when handling specimens and reagents during procedure setup with PROmate™ COVID-19:

- Do not handle reactions tubes by their base as this may affect optical reading. Reaction tubes should be held with gloved hands by the rim/lid to avoid any contact with the sides of the tube
- Tubes must not rest directly on the workbench; use racks for holding tubes wherever possible
- Ensure contents of reaction tubes are at the bottom of wells prior to addition of sample, and prior to running on the instrument
- Ensure absence of any bubbles in reaction tubes prior to running on the instrument. Failure to do so could result in errors in signal interpretation by the instrument
- **Before** opening the positive control pouch, visually inspect for leakage. If there is evidence of leakage inside the bag, **DO NOT OPEN** and use a replacement positive control to avoid contamination
- Visually inspect all qPCR reaction tubes prior to use for any evaporative losses. Upon defrosting of components, collecting the liquid at the bottom of the qPCR reaction tubes (by flicking/spinning) will show any tubes which have reduced volumes. These should **NOT** be used and instead replaced with full, inspected qPCR reaction tubes prior to running PROmate™

For total user flexibility there are **two separate** workflows for using PROmate™ COVID-19:

- Processing of samples **outside** of a Category II biosafety cabinet – protocol in **section 13.2**
- Processing of samples **with** a Category II biosafety cabinet– protocol in **section 13.3**

Please follow the appropriate protocol for your workflow stream below. It is important the correct workflow is followed to ensure user safety and total viral inactivation before sample manipulation prior to PCR setup.

13.2 Swab Specimen Processing outside of a Category II biosafety cabinet

Sample Collection

a) Add one lyophilised RNase inhibitor cake with red bead to the Sample Preparation Buffer tube to be used. The bead within is coloured red; this provides easy identification that the inhibitor has been added.
b) Place patient swab in assigned Sample Preparation Buffer tube and break to fit inside the tube (**optional:** use scissors if necessary to cut swab to length appropriately). Close lid with swab inside.
c) ***Barcode users only (if not, label appropriately):*** Scan the barcode on the Sample Preparation Buffer tube to link the patient to the sample ID. Manual users should label the sample appropriately to ensure patient sample traceability.
d) Repeat steps a & b) for all patient samples to be processed. If using scissors, ensure they are decontaminated with 70% alcohol (ethanol or isopropanol) in between each sample.
e) Once all samples have been resuspended, leave the Sample Preparation Buffer tubes at room temperature for at least **5 minutes**. This is necessary for total SARS-CoV-2 inactivation.

Lab processing

f) Open frozen PROmate™ Pack and defrost magazine with PCR reaction tubes (including negative control tube). Ensure liquid content is at the bottom of the tubes.
g) Defrost positive control tube from frozen PROmate™ Pack. DO NOT open pouch.
h) Defrost negative control solution tube from frozen PROmate™ Pack.
i) Vortex each Sample Preparation Buffer tube thoroughly to release swab contents into solution.
j) Once ambient incubation is complete, remove the foil on a qPCR reaction tube and transfer 5µl of the relevant patient Sample Preparation Buffer tube into the designated qPCR reaction tube. Seal with provided cap. **Flick to remove any bubbles.**
k) Repeat step j) for all samples.
l) Add 5µl of the Negative Control Solution (red lid) into the PCR reaction negative control tube (position N) and seal. **Flick to remove any bubbles.**
m) Inspect, and if no leakage present, open the Positive Control pouch, remove the tube and ensure the liquid content is at bottom of the tube. Place in position "P" of the magazine. **Flick to remove any bubbles**. DO NOT open the Positive control qPCR tube.
n) Place magazine in correct orientation to q16/q32. If operating with a partial run (i.e. NOT complete magazines), use blank 0.1ml qPCR tubes to fill gaps and balance the lid on the instrument.
o) ***Barcode scanner users only (if no barcode scanner, skip this step):*** Using the supplied "genesig q16 Sample Queue Software" Scan the magazine ID and the patient samples, assigning to the correct wells. Assign the Negative Control to position 15 and the Positive Control to position 16 (as indicated)
p) If using the q16, start "PROmate kit (manual setup)" for manual users, or if using the barcode scanner, click "PROmate kit (auto setup from q16 Sample Queue app)". These protocols are on software v.2.10.5. If using the genesig® q32, start the run protocol using the provided template file.
q) ***Barcode scanner users only (if no barcode scanner, input instrument manually):*** Scan the relevant instrument barcode when asked to select the instrument upon which the run will take place. Manual users should take care to select the correct instrument for operation where multiple instruments are available.

13.3 Swab Specimen Processing with a Category II biosafety cabinet

a) Add one lyophilised RNase inhibitor cake with red bead to each Sample Preparation Buffer tube to be used, close and invert to mix. The bead within is coloured red; this provides easy identification that the inhibitor has been added.
b) Place patient swab in assigned Sample Preparation Buffer tube and twirl to release the swab contents into suspension. Upon removal of swab, squeeze head against side of tube to release liquid. Close lid and vortex thoroughly.
c) ***Barcode users only (if not, label appropriately):*** Scan the barcode on the Sample Preparation Buffer tube to link the patient to the sample ID. Manual users should label the sample appropriately to ensure patient sample traceability.
d) Repeat step b) for all patient samples to be processed.
e) Once all samples have been resuspended, leave the Sample Preparation Buffer tubes at room temperature for at least **5 minutes**. This is necessary for total SARS-CoV-2 inactivation.
f) Open frozen PROmate™ Pack and defrost magazine with PCR reaction tubes (including negative control tube). Ensure liquid content is at the bottom of the tubes.
g) Defrost positive control tube from Pack 2. DO NOT open pouch.
h) Defrost negative control solution tube from frozen PROmate™ Pack.
i) Once ambient incubation is complete samples can be handled outside of the Category II biosafety cabinet. Remove the foil on a qPCR reaction tube and transfer 5µl of the relevant patient Sample Preparation Buffer tube into the designated qPCR reaction tube. Seal with provided cap. **Flick to remove any bubbles or use microcentrifuge.**
j) Repeat step i) for all samples.
k) Add 5µl of the Negative Control Solution (red lid) into the PCR reaction negative control tube (position N) and seal. **Flick to remove any bubbles or use microcentrifuge.**
l) Inspect, and if no leakage present, open the Positive Control pouch, remove the tube and ensure the liquid content is at bottom of the tube. Place in position "P" of the magazine. **Flick to remove any bubbles or use microcentrifuge**. DO NOT open the Positive control qPCR tube.
m) Place magazine in correct orientation to q16/q32. If operating with a partial run (i.e. NOT complete magazines), use blank 0.1ml qPCR tubes to fill gaps and balance the lid on the instrument.
n) ***Barcode scanner users only (if no barcode scanner, skip this step):*** Using the supplied "genesig q16 Sample Queue Software" Scan the magazine ID and name the patient samples, assigning to the correct wells. Assign the Negative Control to position 15 and the Positive Control to position 16 (as indicated)
o) If using the q16, start "PROmate kit (manual setup)" for manual users, or if using the barcode scanner, click "PROmate kit (auto setup from q16 Sample Queue app)". These protocols are on software v.2.10.5. If using the genesig® q32, start the run protocol using the provided template file.
p) ***Barcode scanner users only (if no barcode scanner, input instrument manually):*** Scan the relevant instrument barcode when asked to select the instrument upon which the run will take place. Manual users should take care to select the correct instrument for operation where multiple instruments are available.

Note: PROmate COVID-19 can be used with previously extracted samples (See Appendix I)

13.4 Programming the Real-Time PCR Instrument

Please refer to one of the following manuals for additional information on using the instrument:

- genesig® q16 (Primerdesign, Novacyt, software version 2.10.5). Cycling conditions are preloaded in the software
- genesig® q32 (Primerdesign, Novacyt, software version 1.2.2). Cycling conditions are provided in template run file: [PROmate COVID-19]

14. Interpretation of Results Using PROmate™ COVID-19

14.1 Acceptance Criteria of Controls on genesig® q16

The genesig® q16 has automatic calling software which calculates the result and presents this to the user through a simple interpretation. If the following criteria are not satisfied, then testing needs to be repeated:

a) Negative Control is blank, e.g., there should be no status in this well.
b) Positive Control is "Pass"

14.2 Interpretation of Patient Specimen Results using genesig® q16

Patient Specimen Results are interpreted by the genesig® q16 software and displayed as

a) If a patient specimen is negative, it will display "Negative" in red
b) If a patient specimen is positive, it will display "Positive" in green
c) If a sample IC has not passed acceptance criteria, "inconclusive" will display and a repeat of that patient sample should be processed

14.3 Acceptance Criteria of Controls on genesig® q32

Before interpreting sample results, it is necessary to verify the success of the run. If the following criteria are not satisfied, then testing needs to be repeated:

a) Negative Control produces positive amplification in the HEX channel (this is detection of the RNA Internal Control) and is free from amplification in the FAM channel***
b) PCT produces a Cq of between 14-25 in the FAM channel

*** If the Negative Control does produce positive amplification in the FAM channel, the FAM Cq value produced by the patient sample should be >5Cq earlier than the FAM Cq value of the Negative Control (e.g., patient sample FAM Cq = 30, Negative Control FAM Cq ≥35 is acceptable) in order to proceed with the interpretation of patient specimen results using the genesig® q32 (section 14.4)

If the patient sample produces a FAM Cq <5Cq earlier than the Negative control Cq (e.g patient sample FAM Cq = 30, Negative Control FAM Cq = 32) results should not be analysed due to contamination.

If any patient sample produces a FAM Cq >37.66 the result should be deemed negative, if all quality control inputs are satisfied, as amplification is due to contamination.

When using the genesig® q32, please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

14.4 Interpretation of Patient Specimen Results using genesig® q32

If all the control acceptance criteria are fulfilled, then each sample can be assessed with the below criteria if using PROmate™ COVID-19 with the genesig® q32.

		SARS-CoV-2 Target (FAM (465-510))	
		Cq Positive	Cq Negative
IC Target (HEX (533-580))	Cq Positive	SARS-CoV-2 Positive*	SARS-CoV-2 Negative**
	Cq Negative	SARS-CoV-2 Positive*	Result invalid Repeat testing of sample

*All instances of test sample amplification in the FAM channel indicate a SARS-CoV-2 positive sample. Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

**If there is no amplification in the FAM channel for a test sample, to confirm the FAM result is valid as SARS-CoV-2 negative, there should be an amplification Cq < 22 in the HEX channel on the IC. This confirms the PCR run is valid.

15. Limitations of the procedure

- The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results.
- Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- As with any molecular test, mutations within the target sequence of SARS-CoV-2 could affect the PROmate™ COVID-19 primer and/or probe binding, resulting in failure to detect the presence of the virus.
- False negative results may be caused by:
    - Unsuitable collection, handling and/or storage of samples.
    - Sample outside of viraemic phase.
    - Failure to follow procedures in this handbook.
- False positive results may be caused by:
    - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
    - Unsuitable handling of amplified product.
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.

16. Performance Evaluation

16.1 Analytical Sensitivity

The limit of detection (LoD) is defined as the lowest concentration of the analyte that can be reliability detected with >95% confidence. The LoD of PROmate™ COVID-19 was validated by testing at least 20 anterior nasal swab samples provided from 5 different donors on both the genesig® q16 Real Time PCR system and the genesig® q32 Real Time PCR system. Each biological replicate was contrived with SARS-CoV-2 whole genome RNA provided by Twist Bioscience® (MT007544.1).

Sample contrivance was performed after the viral inactivation step to represent the sensitivity of the assay to detect the released viral RNA.

PROmate™ COVID-19 LOD is defined as 0.96copies/ul, or 960 copies/ml. The limit of detection was calculated using SARS-CoV-2 whole genome RNA provided by Twist Bioscience® (MT007544.1). The results are summarised below:

The contrivance level achieved with the prepared sample using Twist Bioscience® SARS-CoV-2 RNA had a starting concentration of 6x10^3 copies/ml, which was then diluted accordingly.

genesig® q16 Real Time PCR system					
Sample type	**SARS-CoV-2 Viral RNA Concentration (copies/µl in PCR reaction)**	**Positive calls/Total no. results included in analysis**	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
Anterior nasal swab	0.96	58/60	96.67	35.57	0.93
Anterior nasal swab	0.88	50/60	83.33	35.82	0.89
Anterior nasal swab	0.80	18/20	90.00	36.17	0.98

genesig® q32 Real Time PCR system					
Sample type	**SARS-CoV-2 Viral RNA Concentration (copies/µl in PCR reaction)***	**Positive calls/Total no. results included in analysis**	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
Anterior nasal swab	0.88	40/40	100.0	34.76	1.02

The LoD for PROmate™ COVID-19 is therefore defined as 0.96copies/ul, or 960 copies/ml.

16.2 Oropharyngeal swab validation

The objective of this bridging study is to show the non-inferiority of Oropharyngeal swabs compared to anterior nasal swabs on PROmate™ COVID-19 (Direct to PCR) using the genesig® q32 real time PCR instrument. This is done by demonstrating that the analytical sensitivity (Limit of Detection) of PROmate™ COVID-19 workflow for Oropharyngeal swabs is equivalent to anterior nasal swabs (0.96 copies/µl). The Limit of Detection (LoD) is defined as the lowest concentration of analyte that can be detected with >95% confidence.

Overall, the LoD obtained from the Oropharyngeal swabs was the same as the anterior nasal swabs (0.96 copies/µl).

genesig® q32 Real Time PCR system					
Sample type	**SARS-CoV-2 Viral RNA Concentration (copies/µl in PCR reaction)***	**Positive calls/Total no. results included in analysis**	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
Anterior nasal swab	0.96	40/40	100.0	34.76	1.02
Oropharyngeal swab	0.96	20/20	100.0	36.20	0.43

*Exsig Direct equivalent is used for reporting.

16.2.1 Analytical Sensitivity post extraction

This study demonstrated that PROmate COVID-19 could be used on previously extracted samples (see appendix I and II).

Saliva samples (negative for SARS-CoV-2) were contrived with whole genome SARS-CoV-2 RNA (Twist Bioscience®) and extracted using the Kingfisher Flex automated extraction platform in conjunction with the exsigTM Mag extraction kit (Primerdesign). Eluates were run with the PROmateTM COVID-19 assay on the genesig® q16 qPCR platform (see appendix I for workflow).

Overall, the LoD obtained from PROmate used with extracted samples was 0.63 copies/µl.

Genesig q16 Real Time PCR system					
Total Copies Contrived in Sample (100 µl)	**SARS-CoV-2 Viral RNA Concentration (copies/µl in PCR reaction)**	**Positive calls/Total no. results included in analysis**	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
250	0.63	19/20	95	35.2	1.2

16.3 Accuracy

Diagnostic accuracy of the PROmate™ COVID-19 was determined by generating a Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and Overall Percentage Agreement (OPA). Samples were tested blind with PROmate™ COVID-19 and compared with the contrivance status (positive or negative) to produce the percentage agreements.

Alongside this PROmate™ COVID-19 accuracy study, a comparison accuracy study was performed between PROmate COVID-19 and an alternative direct PCR assay: exsig® COVID-19 Direct. The PPA, NPA and OPA of each kit was calculated and compared to the alternative kit.

Anterior nasal dry swabs were collected from 8 donors to make a total of 150 biological samples. 50 samples were contrived at 3 x the LoD, as defined in **Section 16.1**, to produce positive samples. Contrivance was conducted with SARS-CoV-2 RNA provided by Twist Bioscience® (MT007544.1). The remaining 100 samples were not contrived and remained negative. The below tables show the results summary:

PROmate™ COVID-19:

Results for the blind contrivance accuracy study using PROmate™ COVID-19

		Sample contrivance status		
		Positive	Negative	Total
PROmate™ COVID-19	Positive	50	4	54
	Negative	0	96	96
	Total	50	100	150

Agreement	Level
OPA	97%
PPA	100%
NPA	96%

exsig® COVID-19 Direct:

Results for the blind contrivance accuracy study using exsig® COVID-19 Direct

		Sample contrivance status		
		Positive	Negative	Total
Comparator exsig® COVID-19 Direct	Positive	49	8	57
	Negative	1	92	93
	Total	50	100	150

Agreement	Level
OPA	94%
PPA	98%
NPA	92%

The above data sets summarises the total agreement on blind contrived samples tested on

PROmate™ COVID-19 and exsig™ COVID-19 Direct.

16.3.1 Accuracy post extraction

Diagnostic accuracy of the PROmate™ COVID-19 when used with previously extracted samples, was determined by generating a Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and Overall Percentage Agreement (OPA). Samples were tested blind with PROmate™ COVID-19 and compared with the contrivance status (positive or negative) to produce the percentage agreements (see Appendix I and II).

PROmate™ COVID-19 (with extracted samples):
Results for the accuracy study using PROmate™ COVID-19 with previously extracted samples.

		Sample Contrivance status		
		Positive	Negative	Total
PROmate COVID-19 post extraction samples	Positive	30	1	31
	Negative	0	105	105
	Total	30	106	136

Agreement	Level
PPA	97%
NPA	100%
OPA	99.3%

Comparator assay
Results for the accuracy study performed with previously extracted samples using a comparator assay.

		Sample Contrivance status		
		Positive	Negative	Total
RespiBio® Multiplex PCR Assay (Serosep).	Positive	31	0	31
	Negative	0	105	105
	Total	30	105	136

Agreement	Level
PPA	100%
NPA	100%
OPA	100%

*Investigation of discrepant result from 1/136 samples tested, revealed this known positive found to be negative by PROmate™ method, was only weakly positive with the comparator assay (Cq 37.25). It is likely that this represents a sample with levels of viral RNA nearing the detection limit of molecular assays and has potentially undergone degradation since the original testing with the comparator assay.

16.4 Analytical specificity

16.4.1. Inclusivity

To ensure the PROmate™ COVID-19 primers and probe remain specific to detect SARS-CoV-2 genomes, Primerdesign's Bioinformaticians review daily the SARS-CoV-2 sequence submissions on the GISAID EpiCoV database. As of 21st of December 2020, *in silico* analysis confirms the COVID-19 assay primers and probes for the ORF1ab target within show 100% detection with the 192,218 full length, good quality SARS-CoV-2 sequences published on the GISAID EpiCoV database.

16.4.2. Exclusivity

Related Pathogens and pathogens that are likely to be present in the clinical specimen have been evaluated *in silico* to identify the homology between the primers/probe of the assay and the pathogens. Upon *in silico* analysis, the genesig® Real-Time PCR COVID-19 (CE IVD) assay exhibited no cross-reactivity with non-SARS-CoV-2 species except for two sequences, Bat coronavirus (NCBI Accession No. MN996532.1) and Pangolin coronavirus (NCBI Accession No. MT084071.1) sequences.

In vitro testing:

For *in vitro* testing, 4 panels were sourced:

- Respiratory Evaluation Panel (Qnostics, Scotland, UK)
- QCMD panel from the 2019 Coronavirus EQA programme (Qnostics)
- Respiratory validation panel (ZeptoMetrix)
- Pneumonia Validation panel (ZeptoMetrix)

The samples from these panels are representative of true clinical human specimens and evaluated in triplicates. The results of the *in vitro* cross-reactivity testing are presented below:

Virus	Strain	Source	Detected/Replicates	Final result
INF A H1N1 positive	-	Isolate	0/3	Negative
INF A H3N2 positive	-	Isolate	0/3	Negative
INF B Victoria	-	Isolate	0/3	Negative
INF B Yamagata	-	isolate	0/3	Negative
RSV A	-	isolate	0/3	Negative
RSV B	-	isolate	0/3	Negative
Coronavirus	NL63	isolate	0/3	Negative
Coronavirus	229E	isolate	0/3	Negative
Coronavirus	HKU	isolate	0/3	Negative
Coronavirus	OC43	isolate	0/3	Negative
Influenza AH1	-	isolate	0/3	Negative
Influenza AH3	-	isolate	0/3	Negative
Influenza B		isolate	0/3	Negative
Metapneumovirus	-	isolate	0/3	Negative
Enterovirus	-	isolate	0/3	Negative
Adenovirus 3	-	isolate	0/3	Negative
Parainfluenza 3	-	isolate	0/3	Negative
Rhinovirus	-	isolate	0/3	Negative
S. pyogenes	Z018	isolate	0/3	Negative
Parainfluenza 2	-	isolate	0/3	Negative
S. pneumoniae	Z022	isolate	0/3	Negative
S. marcescens	Z053	isolate	0/3	Negative
S. aureus	MRSA, COL	isolate	0/3	Negative
S. agalactiae	Z019	isolate	0/3	Negative
K. pneumoniae	Z460; NDM-1	isolate	0/3	Negative
Coronavirus SARS	-	isolate	0/3	Negative
Parainfluenza	-	isolate	0/3	Negative
K. pneumoniae	Z138	isolate	0/3	Negative
K. pneumoniae	Z460	isolate	0/3	Negative
P. aeruginosa	Z139, VIM1	isolate	0/3	Negative
P. mirabilis	Z050	isolate	0/3	Negative
K. aerogenes	Z052	isolate	0/3	Negative
H. influenzae	MinnA	isolate	0/3	Negative
E. coli	Z297	isolate	0/3	Negative
E. cloacae	Z101	isolate	0/3	Negative
A. baumannii	307-0294	isolate	0/3	Negative

16.5 Interfering substances

The possible interference of exogenous and endogenous substances present in anterior nasal samples on the PROmate™ COVID-19 assay performance was analysed by identifying significant changes in the Cq values, in samples containing these potential interfering substances. When an interferent effect was observed, a further dose response study was performed on the specific substance to establish the extent of this change in the assay performance.

The interfering substances considered in the study are shown below:

- Nasal corticosteroid
- Nasacort
- Blood
- Tobramycin
- Guafinesin
- Dexamethasone
- Oseltamivir
- Oxymetazoline
- Mupirocin
- Fluticasone
- Mucin

For each interfering substance, saliva sample negative for COVID-19 was collected from the same donor. In parallel, a set of interfering substances controls (ISC) containing a COVID-19 saliva sample but not interfering substance were extracted.The appropriate volume of interfering substance (IS) at the relevant concentration (see table below) was contrived into each sample before the extraction phase.

Interfering substance	Neat Concentration		Volume of IS to be added to the sample		Volume of solvent to be added to the sample		Final concentration in study		Control
Nasal corticosteroid	137.0	mg/ml	50.0	µl	0.0	µl	6.493	mg/ml	Water
Nasacort	100	%	50.0	µl	0.0	µl	4.739	%	Water
Blood	10.0	g/ml	2.1	µl	47.9	µl	0.02	g/ml	Water
Tobramycin	0.6	mg/ml	50.0	µl	0.0	µl	0.028	mg/ml	Water
Guafinesin	60.0	mg/ml	50.0	µl	0.0	µl	2.844	mg/ml	5% Ethanol
Dexamethasone	30.6	µmol/l	50.0	µl	0.0	µl	1.450	µmol/l	5% Ethanol
Oseltamivir	2.0	mg/ml	50.0	µl	0.0	µl	0.095	mg/ml	Water
Oxymetazoline	0.2	mg/ml	50.0	µl	0.0	µl	0.009	mg/ml	Water
Mupirocin	30	µg/ml	50.0	µl	0.0	µl	1.422	µg/ml	16% Ethanol
Fluticasone	1.0	mg/ml	50.0	µl	0.0	µl	0.047	mg/ml	10% Ethanol
Mucin	0.4	mg/ml	50.0	µl	0.0	µl	0.190	mg/ml	5% 1M NaOH

The results obtained are as follow:

	Interfering Substance			Control			
Channel	Substance	Mean Cq	SD	Control	Mean cq	SD	Cq difference
FAM	Nasal corticosteroid	33.4	0.21	Water	33.8	1.03	-0.40
VIC		17.0	0.50		17.0	0.00	-0.03
FAM	Nasacort	33.4	0.31		33.8	1.03	-0.40
VIC		17.0	0.13		17.0	0.00	-0.06
FAM	Blood	34.2	0.48		33.9	0.84	0.30
VIC		17.2	0.13		17.2	0.10	0.01
FAM	Tobramycin	33.3	0.76		33.9	0.84	-0.60
VIC		17.2	0.17		17.2	0.10	0.03
FAM	Guafinesin	33.4	0.50	5% Ethanol	33.9	0.49	0.50
VIC		16.6	0.70		17.2	0.13	0.70
FAM	Dexamethasone	34.2	0.49		33.9	0.49	0.50
VIC		17.1	0.10		17.2	0.13	0.09
FAM	Oseltamivir	33.5	0.52	Water	33.5	0.40	0.05
VIC		16.6	0.89		17.1	0.05	-0.70
FAM	Oxymetazoline	33.5	0.39		33.9	0.40	0.08
VIC		17.1	0.13		17.8	0.05	-0.25
FAM	Mupirocin	34.1	0.40	16% Ethanol	34.0	0.60	0.20
VIC		17.7	0.05		17.8	0.15	-0.20
FAM	Fluticasone	34.6	0.41	10% Ethanol	33.7	0.39	0.90
VIC		18.0	0.13		17.3	0.08	0.60
FAM	Mucin	34.6	0.43	5% 1M NaOH	33.4	0.43	0.80
VIC		18.2	0.90		17.8	0.83	0.40

None of the substances studied affected the PCR reaction of the PROmate™ COVID-19 kit negatively. The standard deviations were always within 1 Cq of the appropriate control used in the run.

16.6 Precision

The precision study was performed as part of the Analytical sensitivity study. Three batches of PROmate COVID-19 (CE-IVD) were tested across two days. Inter batch reproducibility was demonstrated, and the LoD was maintained across the 3 batches of PROmate COVID-19 tested with >95% confidence with a standard deviation of 0.93. The intra batch repeatability was shown and the standard deviations obtained were as follows: Batch 1: 1.07, batch 2: 0.77 and batch 3: 0.79.

16.7 Appendix I

Alternative workflow for using PROmate COVID-19 with post extraction samples.

a) Open frozen PROmate™ Pack and defrost magazine with PCR reaction tubes (including negative control tube). Ensure liquid content is at the bottom of the tubes.
b) Defrost positive control tube from Pack 2. DO NOT open pouch.
c) Defrost negative control solution tube from frozen PROmate™ Pack.
d) Remove the foil on a qPCR reaction tube and transfer 5µl of previously extracted RNA into the designated qPCR reaction tube. Seal with provided cap. Flick to remove any bubbles or use microcentrifuge.
e) Repeat step d) for all samples.
f) Add 5µl of the Negative Control Solution (red lid) into the PCR reaction negative control tube (position N) and seal. Flick to remove any bubbles or use microcentrifuge.
g) Inspect, and if no leakage present, open the Positive Control pouch, remove the tube and ensure the liquid content is at bottom of the tube. Place in position "P" of the magazine. Flick to remove any bubbles or use microcentrifuge. DO NOT open the Positive control qPCR tube.
h) Place magazine in correct orientation to q16/q32. If operating with a partial run (e.g., NOT complete magazines), use blank 0.1ml qPCR tubes to fill gaps and balance the lid on the instrument.
i) Barcode scanner users only (if no barcode scanner, skip this step): Using the supplied "genesig q16 Sample Queue Software" Scan the magazine ID and name the patient samples, assigning to the correct wells. Assign the Negative Control to position 15 and the Positive Control to position 16 (as indicated) o) If using the q16, start "PROmate kit (manual setup)" for manual users, or if using the barcode scanner, click "PROmate kit (auto setup from q16 Sample Queue app)". These protocols are on software v.2.10.5. If using the genesig® q32, start the run protocol using the provided template file.
j) Barcode scanner users only (if no barcode scanner, input instrument manually): Scan the relevant instrument barcode when asked to select the instrument upon which the run will take place. Manual users should take care to select the correct instrument for operation where multiple instruments are available.

See above for instructions regarding Programming the Real-Time PCR Instruments.

Interpretation of results can be found in section 14 above.

16.8 Appendix II

Performance evaluation Post extraction workflow.

Analytical Sensitivity

This study established the analytical parameters (LOD) of the PROmate™ COVID-19 assay on the genesig® q16 qPCR platform when Saliva samples (negative for SARS-CoV-2) were contrived with whole genome SARS-CoV-2 RNA (Twist Bioscience®) and extracted using the Kingfisher Flex automated extraction platform in conjunction with the exsig™ Mag extraction kit (Primerdesign).

LOD was determined by testing 3 different contrivance levels of SARS-CoV-2 RNA (20 biological replicates each). The lowest contrivance level that produced at least a 95% detection rate was selected as the LOD for this assay.

Analyte Contrivance Level	Total Copies Contrived in Sample (100 µl):	Concentration: Copies/reaction	Concentration: Copies/µl in the reaction
1	250	12.5	0.63
2	125	6.25	0.31
3	62.5	3.125	0.156

Table 1. Contrivance levels used in this study.

Contrivance Level	SARS-CoV-2 Target Orf1ab			Internal Control		
	n detected	Mean Cq	Detection Rate	n detected	Mean Cq	Detection Rate
1	19/20	35.2	95%	20	17.8	100%
2	14/20	35.4	70%	17	17.9	85%
3	8/20	35.7	40%	20	17.7	100%

Table 2. LOD verification data for the 3 contrivance levels tested.

Contrivance Level 1 met the required 95% detection rate for LOD verification (Table 1). Therefore, LOD was verified at Contrivance level 1, 0.625 copies/µl in the qPCR reaction (12.5 copies/reaction).

Clinical Performance evaluation

Clinical evaluation of the PROmate COVID-19 post extraction workflow was conducted with RNA extracted on the LifeRiver Ex3600. Results of PCR were compared to the SARS-CoV-2 result of the comparator assay, the RespiBio® Multiplex PCR Assay (Serosep).

105 nasal/Oropharyngeal swabs previously designated negative by comparator assays were re-tested retrospectively using the PROmate™ COVID-19 assay on the genesig® q32 Real Time PCR machine (see Appendix I for workflow). The negative results were confirmed with downstream comparator assays in all cases, indicating concordance of results.

31 nasal/Oropharyngeal samples previously designated positive by comparator assays were re-tested retrospectively using the PROmate™ method. Amongst these samples, 8 were strong positives (Cq 15-25), 16 were medium positives (Cq ≥25-30), and 7 were weak positive (Cq >30) by the comparator assay. Of the 31 known positive specimens, 30 were confirmed to be positive by the PROmate™ assay. The remaining 1 nose and Oropharyngeal swab tested NEGATIVE indicating a discrepant result.

PROmate™ COVID-19 (with extracted samples):

Results for the accuracy study using PROmate™ COVID-19 with previously extracted samples.

		Sample Contrivance status		
		Positive	Negative	Total
PROmate COVID-19 post extraction samples	Positive	30	1	31
	Negative	0	105	105
	Total	30	106	136

Agreement	Level
PPA	97%
NPA	100%
OPA	99.3%

Comparator assay

Results for the accuracy study performed with previously extracted samples using a comparator assay.

		Sample Contrivance status		
		Positive	Negative	Total
RespiBio® Multiplex PCR Assay (Serosep).	Positive	31	0	31
	Negative	0	105	105
	Total	30	105	136

Agreement	Level
PPA	100%
NPA	100%
OPA	100%

*Investigation of discrepant result 1/136 samples tested revealed this known positive found to be negative by PROmate™ method, was only weakly positive with the comparator assay (Cq 37.25). It is likely that this represents a sample with very low levels of viral RNA that is nearing the detection limit of molecular assays and has undergone degradation since the original testing with the comparator assay.

17. Disposal

Dispose of unused kit reagents, human specimens and sealed post-amplification plates as laboratory clinical waste according to national regulations. Refer to **Section 8** for guidance weblinks.

The PROmate™ Sample Preparation Buffer contains Triton X 100 Reduced and is very toxic to aquatic life with long lasting effects. Do not let product enter drains and discharge into the environment must be avoided.

18. Primerdesign Ltd Quality Control

In accordance with Primerdesign Ltd ISO 13485 certified Quality Management System, each batch of the PROmate™ COVID-19 assay is tested against predetermined specifications to ensure consistent product quality.

Primerdesign Ltd perform weekly *in silico* analysis of all published SARS-CoV-2 genomes (GISAID EpiCoV and NCBI databases) to identify if the virus mutates in the COVID-19 primer and probe target region.

19. Technical Support

For Technical support, please contact our dedicated technical support team on:

Phone: +44 (0) 800 0156 494

Email: support@primerdesign.co.uk

20. Trademarks and Disclaimers

Trademarks: PROmate™, genesig® and the Primerdesign logo.

All other trademarks that appear in this IFU are the property of their respective owners.

21. Explanation of Symbols

Symbol	Explanation
	In Vitro Diagnostics
	Manufacturer
	Catalogue number
	Suffices for
	Use by Date
	Temperature limit
	Consult Electronic Instructions for Use
	Batch Code
	Single Use
	Keep away from sunlight (primer/probe mix)
	Positive Control
	EU Authorized Representative

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