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Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay

For Emergency Use Authorization Only

Instructions for Use (IFU) *Issue 1.02*

For *In-vitro* Diagnostic (IVD) Use

Rx Only

GENESIG
Kits by Primerdesign

Primerdesign Ltd COVID-19 genesig® Real-Time PCR Assay

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

For Use with:

Sample Types	Extraction Platforms	PCR Platform
Nasopharyngeal Swabs	GXT DNA/RNA Extraction kit (GenoXtract®, Bruker-HAIN Lifescience GmbH) QIAamp® Viral RNA Mini kit (Qiagen extraction system)	Applied Biosystems® 7500 Real-Time PCR System Bio-Rad CFX Connect™ Real-Time PCR Detection System Roche® LightCycler 480 II
Oropharyngeal Swabs		
Bronchoalveolar Lavage		

Σ 96 tests

For Emergency Use Authorization Only

Rx only

IVD

REF Z-COVID-19 (US ONLY)

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GENESIG

Contents

1. Intended Use

The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, oropharyngeal swab specimens, and bronchoalveolar lavage from patients suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 is generally detectable in nasal, oropharyngeal swab specimens, and bronchoalveolar lavage during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organisation (WHO) in December 2019. Chinese authorities identified a novel coronavirus SARS-CoV-2 (COVID-19 previously called 2019-nCoV) which has resulted in confirmed human infections worldwide, including the United States. Cases of severe respiratory illness and deaths have been reported. Patients can become infected with SARS-CoV-2 virus by person-person contact (through contact with a contaminated environment or person).

The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is a molecular *in vitro* diagnostic test for the detection of the SARS-CoV-2 RNA in oropharyngeal swab specimens from patients suspected of COVID-19 by their healthcare provider and aids the diagnosis of coronavirus COVID-19 disease. The assay is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes, as well as control material, for the use in reverse transcriptase Real-Time-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in oropharyngeal swab specimens.

3. Principles of the Procedure

The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is an *in vitro* diagnostic test based on Real-Time PCR technology, developed for specific detection of SARS-CoV-2 viral RNA. The probe system is based on the standard hydrolysis probe system known as TaqMan® Technology. The SARS-CoV-2 specific probe is labelled with the FAM fluorophore and the internal control (which is from non-biologically relevant exogenous source) is labelled with the HEX fluorophore.

Real-Time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified RNA. The probes are labelled with fluorescent reporter and quencher dyes.

The oligonucleotide primers and probe for the detection of SARS-CoV-2 were selected from the orf1 ab genome region. The supplied primer/probe mix is designed for the specific detection of SARS-CoV-2 RNA (probe labelled with FAM fluorophore) and the supplied genesig® Easy RNA Internal Extraction control (IEC specific probe is labelled with HEX fluorophore).

RNA isolated and purified from nasopharyngeal swabs, oropharyngeal swabs or bronchoalveolar lavage is reverse transcribed to cDNA and subsequently amplified using one of the three Real-Time PCR instruments: Applied Biosystems 7500 Real-Time PCR System (software version 2.3), or Roche LightCycler 480 II (software version 1.5), or Bio-Rad CFX Connect Real-time PCR Detection System (software 1.1) . 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.

The assay includes an internal extraction control (Genesig® Easy RNA Internal Extraction control), which is added to the IVD nucleic acid extraction system (not provided) to measure RNA extraction purity, detect PCR inhibition and confirm the integrity of the PCR run.

4. Materials Provided

The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay contains:

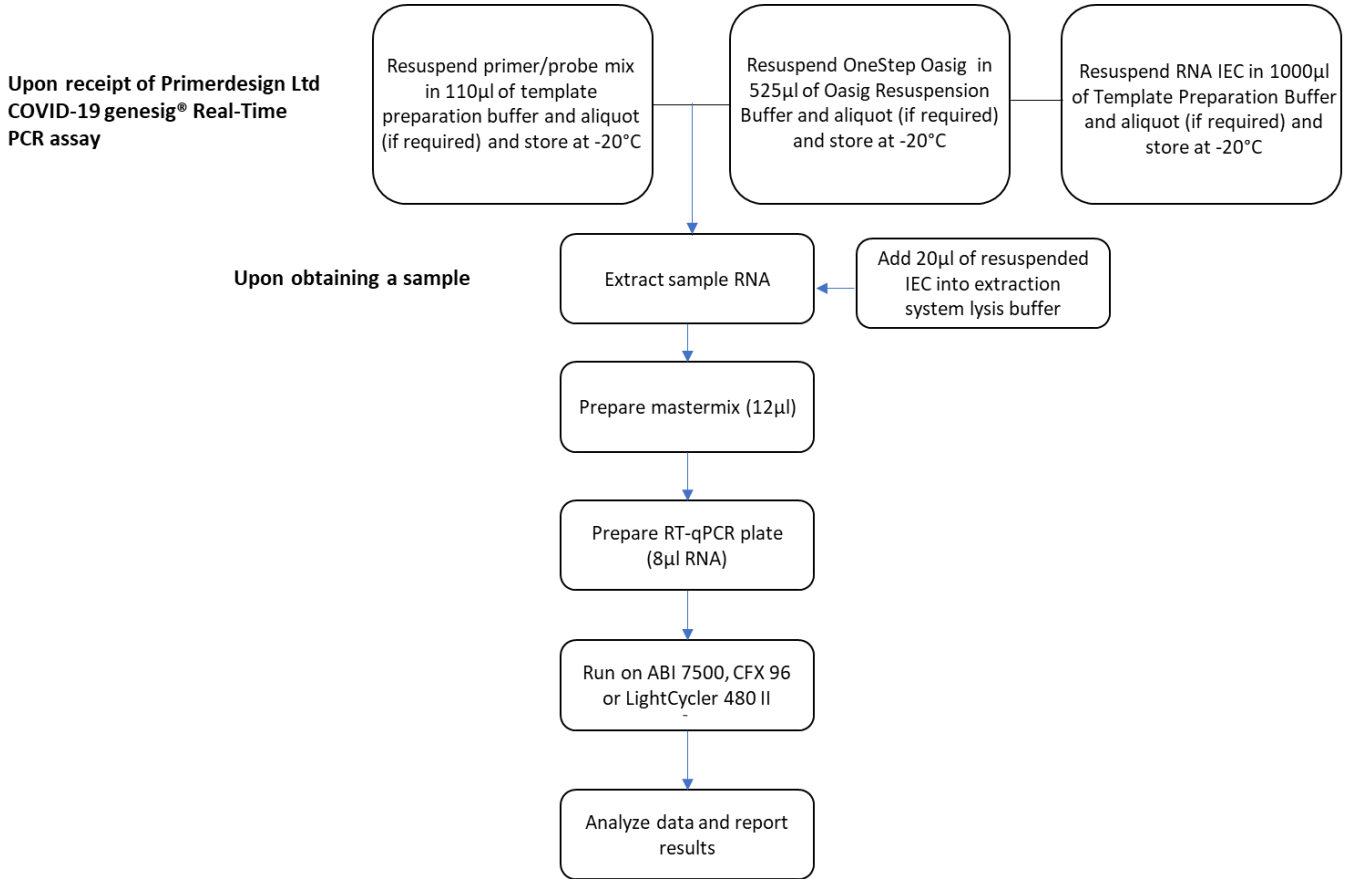
Reagent label	Number of Vials 96 tests	Volume (µL per vial)	Lid colour	Resuspended with?
Oasig™ OneStep 2X RT-qPCR Master Mix Lyophilised	2	525*	Red	Oasig™ resuspension buffer
COVID-19 Primer & Probe Mix (including IEC primer/probe mix)	2	110*	Amber	Template preparation buffer
Oasig™ resuspension buffer	2	750	Blue	n/a
Template preparation buffer	2	1500	Yellow	
Water RNase/DNase Free	1	1500	White	
Genesig® COVID-19 Positive control template	1	600*	Red, vial stored in sealed foil pouch	Template preparation buffer
Genesig® Easy RNA Internal extraction control (IEC)	2	1000*	Blue, vial stored in sealed foil pouch	

*The projected volume once resuspended

The COVID-19 Primer & Probe Mix contains the primers and FAM labeled probe specific to SARS-CoV-2, and also includes the primers and HEX labeled probe specific to the Genesig® Easy RNA Internal extraction control (IEC).

The Oasig™ OneStep 2X RT-qPCR Master Mix, COVID-19 Primers & Probes Mix, Genesig® COVID-19 Positive control template and Genesig® Easy RNA Internal extraction control (IEC) are all provided lyophilized. The table above indicates which buffer to use, as well as the volume to add, to resuspend these reagents.

Summary of Preparation and Testing Process



5. Required Equipment and Consumables (Not Provided)

- PCR hood
- Benchtop microcentrifuge
- Vortex mixer
- Adjustable micropipettes (2 or 10 µl, 200 µl and 1000 µl)
- Adjustable multichannel micropipettes (5-50 µl)
- Racks for 1.5 mL microcentrifuge tubes
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- Aerosol barrier pipette tips with filters
- Disposable gloves
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNA*Zap*™ (Ambion, Catalogue no: AM9890), or equivalent
- RNAse Away™ (Fisher Scientific, Catalogue no: 11580095) or equivalent
- 0.2 mL PCR reaction plates. Depending on the Real-Time PCR instrument to be used, the following are recommended, either:
    - White Roche® LightCycler 480 Multiwell plate 96 (Catalogue no: 04729692001)
    - White Bio-Rad CFX96 Hard-Shell® Low-Profile, Thin-Wall, Skirted 96-Well PCR Plates, White Well (Catalogue No: HSP965)
    - Applied Biosystems® MicroAmp® Optical 96-Well Reaction Plate (Catalogue no: 4316813)
- Water RNase/DNase free
- Plate seal

6. Real-Time PCR instruments

The Primerdesign™ Ltd COVID-19 genesig® Real-Time PCR assay is to be used with the following Real-Time PCR instruments:

- Applied Biosystems® 7500 Real-Time PCR System (software version 2.3, catalogue no: 4351104)
- Roche® LightCycler 480 II (software version 1.5, catalogue no: 05015278001)
- Bio-Rad CFX Connect™ Real-Time PCR Detection System (Maestro™ software version 1.1, catalogue no: 1855201,1855195)

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

7. Extraction Kits / Instruments

The Primerdesign™ Ltd COVID-19 genesig® Real-Time PCR assay is to be used with the following extraction systems:

- Automated extraction system GenoXtract® from HAIN Lifescience GmbH (Brucker) using GXT DNA/RNA Extraction kit (Catalogue no: 12.01.02, 96 samples);
- Qiagen extraction system with QIAamp® Viral RNA Mini kit (Catalogue no: 163013348, Qiagen, Germany)

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 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

In addition, refer to the World Health Organization Interim guidance on laboratory biosafety: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: interim guidance, March 2nd, 2020

https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117

9. Warnings and Precautions

9.1. General

- For in vitro diagnostic use (IVD) only.
- For Emergency Use only.
- For prescription use only.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to 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
- 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) biological safety cabinet.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- 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, eye protection 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 material safety data sheet (MSDS) before using this kit, which is available on request.
    - The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay component "Template preparation buffer" contains EGTA. This component should be handled according to the MSDS. In the event of damage to protective packaging, contact Primerdesign for instructions.

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 genesig®COVID-19 positive control template is provided in a sealed foil envelope and contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
    - Maintain separate areas for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
    - 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 (not previously worn) when setting up assays.
    - Change gloves between samples 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 minimize the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Good aseptic technique should always be used when working with nucleic acids.
    - When mixing reagents by pipetting up and down, this should be done with a volume roughly equal to 50% of the total component volume.
    - **DO NOT** use water to resuspend the kit components. Use the appropriate buffers (provided with the kit) as instructed in the table in **Section 4**.
    - Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g. DNA/RNA remover, ethanol, 10% bleach) to minimize risk of nucleic acid contamination.
- RNA should be maintained on a cold block or on ice during preparation and used to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken.
- Dispose of unused kit reagents and human specimens according to local, state and federal regulations.

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.

9.4. Specimen nucleic acid extraction kit/system

- Please consult the relevant Instruction For Use (IFU) and Materials Safety Data Sheet (MSDS), available from the manufacturer, before using your chosen IVD extraction kit/ system.

10. Reagent Storage, Handling and Stability Conditions

10.1. Storage conditions

- The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is shipped at ambient temperatures but must be stored at -20°C upon arrival.
- If the kit's protective packaging is damaged upon receipt, please contact Primerdesign for instructions. Attention should be paid to the "use by" date specified on the pack label and individual tube labels. On this date, the kit should be discarded following the disposal instructions in **Section 19**
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic primer/probe mix from light.
- Primer/probe mix, the enzyme master mix, positive control template and RNA internal extraction control are all delivered lyophilized and must be resuspended in the appropriate, supplied buffer to the correct volume as detailed in the table in **Section 4**.
- Once resuspended, components may be aliquoted into smaller volumes, if required, and are stable for up to one month if stored at -20°C.

10.2. In Use Stability

- The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay should be stored in the original packaging and is stable for up to 12 months once resuspended and stored at -20°C.
- The kit should not be used past the "use by" date as indicated on the pack label and individual tube labels.
- When *in use* the kit components should be returned to the freezer promptly after use to minimize the time at room temperature.
- Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles. Components may be aliquoted into smaller volumes after resuspension, if required.

11. Specimen Collection, Handling and Storage

11.1. Collecting the Specimen

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.

- Refer to 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

- 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. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport medium.

11.2. Transporting Specimens

- Specimens must be package, 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. Store specimens at 2-8ºC and ship overnight. If a specimen is frozen at -70ºC or lower, ship overnight on dry ice.

11.3. Storing Specimens

- Specimens can be stored at 2-8ºC for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70ºC or lower.
- Extracted nucleic acid should be stored at -70ºC or lower.
- Refer to in Section 7 for details regarding Specimen nucleic acid extraction kits/system.

12. Reagent and Controls Preparation

Reagent preparation:

12.1. Oasig™ OneStep 2x RT-qPCR Master Mix (lyophilized) preparation

- Upon receipt, the dried master mix can be stored at -20ºC for up to six months or the expiry date, whichever occurs first.
- Using aseptic technique, resuspend in 525µl of oasig™ resuspension buffer, gently swirl to mix.

- Store at -20ºC. Resuspended master mix is stable for 12 months when stored at -20ºC.
  Freeze/ thaw cycles should be minimized and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.

12.2. COVID-19 and IEC Primer/Probe mix preparation

- Upon receipt, the dried primers/probes mix can be stored at -20ºC for up to six months or the expiry date, whichever occurs first.
- Precautions: this reagent should only be handled in a clean area and not exposed to light.
- Using aseptic technique, resuspend the dried reagent in 110µl (per each vial) of Template preparation buffer and vortex to mix.
- Store at -20ºC. Resuspended primer/probe mix is stable for 12 months when stored at -20ºC.
- Freeze/ thaw cycles should be minimized and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required.
- Store aliquots in the dark and keep away from exposed sunlight. Store aliquots at -20ºC.

Controls preparation:

Quality control requirements must be performed in conformance with local, state and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices refer to 42 CFR 493.1256.

12.3. Genesig® COVID-19 Positive control template preparation

- The genesig®COVID-19 Positive control template (PCT) is provided in a sealed foil envelope and contains a high copy number of templates. It should be opened and processed away from clinical specimens and kit components to avoid cross-contamination.
- The PCT tube contains synthetic DNA representing the SARS-CoV-2 genomic region of interest. Following resuspension, this will be at a concentration of 1.7×10^5 copies per µl.
- Caution: This reagent contains a high copy number of positive control material and should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination of other kit reagents and clinical specimens.
- Upon receipt, the dried PCT can be stored at -20ºC for up to six months or the expiry date, whichever occurs first.
- Store at -20ºC. Resuspended PCT is stable for 12 months when stored at -20ºC.
- Freeze/ thaw cycles should be minimized and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.
- Using aseptic technique, resuspend the dried PCT in 600µl of Template preparation buffer, mix gently.
- Dilute PCT down to a concentration of 1.7 copies/µl:

- Pipette 990µl of DNase/RNase water into two 1.5 ml microcentrifuge tubes. Label as 1 and 2.
- Pipette 90µl of DNase/RNase water into one 1.5ml microcentrifuge tube. Label as 3.
- Transfer 10µl of resuspended PCT tube into Tube 1. Vortex 20 times.
- Transfer 10µl of Tube 1 to Tube 2. Vortex 20 times.
- Transfer 10µl of Tube 2 to Tube 3. Vortex 20 times. Tube 3 contains 1.7 copies/µl of SARS-CoV-2 DNA specific sequence.

- To ensure PCR run validity, the PCT dilution (1.7 copies/µl) should produce amplification in the FAM channel.

12.4. Genesig® Easy RNA Internal extraction control (IEC) preparation

- The genesig® Easy RNA Internal extraction control (IEC) can be added to the nucleic acid extraction system (not provided) to measure RNA extraction purity, detect PCR inhibition and confirm the integrity of the PCR run.
- Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination.
- Upon receipt, the dried IEC can be stored at -20ºC for up to six months or the expiry date, whichever occurs first.
- Using aseptic technique, resuspend the dried IEC in 1000µl of Template preparation buffer, mix gently.
- Store at -20ºC. Resuspended IEC is stable for 12 months when stored at -20ºC.
- Freeze/ thaw cycles should be minimized and not exceed 5 freeze/thaws. The reagent once resuspended can be aliquoted into smaller volumes if required and stored at -20ºC.

12.5. Negative Extraction Control (NEC) preparation

- Prepare at least 1 negative extraction control (NEC) each time RNA is extracted from a clinical specimen or sample.
- The NEC is an extraction with no clinical specimen/sample added, it is prepared by extracting from RNase/DNase free water. IEC is added to extraction system.
- This NEC will serve as the negative control for the entire testing system and to check for contamination during PCR plate set-up.

12.6. No Template Control

- DNase/RNase free water is a provided to use as a No Template control (NTC) if required in addition to the NEC (refer to **Section 12.5)**
- The NTC is used to check for contamination during PCR plate set-up.

13. General Preparation

13.1. Equipment Preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use.
- Decontamination agents should be used such as 5% bleach, 70% ethanol, and DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.
- Nucleic Acid extraction performance of the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with this assay:
    - Automated extraction system GenoXtract® from HAIN Lifescience GmbH (Brucker) using the GXT DNA/RNA Extraction kit.
    - Qiagen extraction system with QIAamp® Viral RNA Mini kit (Qiagen, Germany)
- Manufacturer's recommended procedures are to be followed for sample extraction.

14. Assay Set Up

14.1. Sample Preparation Procedure

Refer to the 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

Prepare at least 1 negative extraction control (NEC) each time an extraction is performed (i.e. an extraction with no clinical specimen/sample added). This NEC will serve as the negative control for the entire testing system.

	Nasopharyngeal swabs	Oropharyngeal swabs	Bronchoalveolar lavage
Collection	Dacron or polyester flocked swabs in viral transport medium	Dacron or polyester flocked swabs in viral transport medium	Sterile container**
Transport temperature*	4˚C	4˚C	4˚C
Short-term storage (pre-extraction)*	4˚C for ≤ 5 days	4˚C for ≤ 5 days	4˚C for ≤ 48 hours
Long-term storage (pre-extraction)*	-70˚C for longer periods	-70˚C for longer periods	-70˚C for longer periods

*These are CDC recommendations. Local regulations pertaining to sample handling may also apply.
**Sample refers to the viral transport medium provided in the sample container serving as the repository.

14.2. RNA extraction

- The results of the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay is dependent upon the amount and quality of template RNA purified from human specimens. Consult the IFU of the extraction system for full usage details.
- Prepare at least 1 negative extraction control (NEC) each time an extraction is performed (i.e. an extraction with no clinical specimen/sample added).
- The genesig® Easy RNA Internal extraction control (IEC) should be resuspended in 1000µl template preparation buffer. It should be incorporated in the extraction as directed by the extraction system IFU. Primerdesign recommends 20µl is added per sample.
- **The internal extraction control should not be added directly to the clinical specimen/ sample before RNA extraction (i.e. before the clinical specimen/sample is mixed with a lysis buffer of the nucleic acid extraction kit/system). Doing so may compromise the testing.**
- Where the IFU provides no specific guidance for the addition of an Internal extraction control or where an automated system does not support the addition of 20µl IEC, please contact Primerdesign for guidance.

GXT DNA/RNA Extraction Kit VER 2.0 (IFU-120102-10)

During the validation studies, the GXT DNA/RNA Extraction Kit VER 2.0 for the GenoXtract® Automated Extraction System was used to conduct the extractions (according to IFU-120102-10). The internal extraction control (20µl) was applied directly to Well 12 (lysis buffer) of the GXT DNA/RNA Extraction Kit cartridge before proceeding with the protocol.

QIAamp® Viral RNA Mini kit (Qiagen, Germany)

During the validation studies, the QIAamp® Viral RNA Mini kit (Qiagen, Germany) was used

to conduct the extractions according to supplier IFU (QIAamp® Viral RNA Mini kit Handbook from January 2020). The internal extraction control (20µl) was applied directly into lysis stage of extraction.

14.3. Master Mix Setup

a) Resuspend the COVID-19 and IEC primer/probe tube in template preparation buffer, 110µl of buffer per tube, vortex to mix.
b) Resuspend the oasig™ OneStep 2X RT-qPCR Master Mix in 525µl oasig™ resuspension buffer, gently swirl to mix.
c) Plate set-up configuration can vary with the number of specimens. A NEC must be included in each plate set-up (refer to **Section 12.5** on how to prepare NEC). NTCs should be included in each plate set-up. A PCT must be included in each plate set-up.
    a. The PCT will be added after all other reagents and samples have been added to the plate.
    b. This will be in an area for handling nucleic acid and away from the NEC, NTC and any clinical specimen/ samples.
    c. This is to prevent plate set-up, reagent and specimen contamination with the PCT.
d) Determine the number of reactions (n) to set up per assay (including NEC, PCT and any NTCs for each plate). It is necessary to make excess reaction mix to allow for pipetting error. Use the following guide to determine volume of reagents to add to the reaction mix:
    1. If number of samples (n) is ≤ 10, then N = n+1
    2. If number of samples (n) is > 10 and ≤ 20, then N = n+2
    3. If number of samples (n) is > 20, then N = n+ 10% of total number of samples
e) In the reagent set-up room clean hood, centrifuge resuspended Master Mix and COVID-19 primers/probes mix for 5 seconds to collect contents at the bottom of the tube.
f) Prepare a reaction mix of the following reagents from resuspended components in a 1.5ml DNase/RNase free tube:

Reaction mix Component	1 x volume required (µl) *
Oasig™ OneStep 2X RT-qPCR Master Mix	10*
COVID-19 and IEC Primer & Probe	2*

*Multiply all numbers by (N). Refer to step (d) above, to ensure there is sufficient reaction mix for all samples, NEC, PCT and NTCs to be tested.

g) Add 12µl into the number of wells required for your testing, in an appropriate 96 well plate for your chosen PCR platform. Include 1 well for the PCT dilution, 1 well for the NEC and 1 well for the NTC for each PCR plate.
h) Add 8µl of the following into the appropriate wells according to your plate setup:
    a. NEC (please refer to **Sections 12.5**)
    b. NTC (please refer to **Sections 12.6**)
i) Cover the entire reaction plate and move the reaction plate to the specimen nucleic

acid handling area.

j) Gently vortex nucleic acid sample tubes for approximately 5 seconds.
k) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
l) Change gloves often and when necessary to avoid contamination.
m) Add 8µl of the RNA/nucleic acid extracted from clinical specimen/sample(s) into the appropriate wells according to your plate setup.
n) Cover the entire reaction plate and move the reaction plate to the positive template control handling area.
o) Add 8µl of PCT (please refer to **Sections 12.3**) into the appropriate well according to your plate set up. Seal the plate with an appropriate seal and place in the instrument.

14.4. Programming the Real-Time PCR Instrument

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

- Applied Biosystems® 7500 Real-Time PCR system Relative Standard curve and comparative CT Experiments (as per Applied Biosystems manual (2010)).
- LightCycler 480 instrument Operator's manual (July 2016, Addendum 4, Software version 1.5)
- Bio-Rad CFX Connect™ Real-Time PCR Detection System Instrument Guide (as per Bio-Rad Laboratories Inc. Manual (2017))

a) Enter the following amplification program:

Steps	Time	Temperature	Cycles	Detection Format
Reverse Transcription	10 min	55°C	1	COVID-19 = FAM (465-510) RNA Internal Extraction Control (IEC) = VIC / HEX / Yellow555 (533-580)
Initial Denaturation (Taq Activation)	2 min	95°C	1	
Denaturation	10 sec.	95°C	45	
Annealing and Extension	60 sec.	60°C*		

*Acquisition must be performed at the end of this stage
When using Roche® LightCycler 480 II please select the following detection format: Dual Color Hydrolysis Probe / UPL Probe
When using the ABI 7500® please select 'none' for the ROX passive reference dye window in the plate set up

b) Ensure wells loaded with clinical sample(s) are designated as "Sample Type - Unknown"; the software will automatically calculate quantities for these wells if amplification occurs
c) Ensure the well loaded with PCT dilution at 1.7 copy/µl is designated as "Sample Type - Standard" and assigned the appropriate concentration (see **Section 12.3**)

15. Interpretation of Results

Expected performance of controls included in the Primerdesign Ltd COVID-19 genesig Real-Time PCR assay

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) NEC is free from amplification in the FAM (465-510) channel Cq (Quantification cycle) not detected
b) NEC is free from amplification in the FAM (465-510) channel. NEC produces a Cq < 30 in the VIC/HEX/Yellow555 (533-580) channel
c) IEC in a patient sample is positive for exogenous target (The IEC Cq value produced by the patient sample should not exceed IEC Cq value + 6 produced by the NEC)
d) PCT dilution at 1.7 copies/µl produces amplification in the FAM (465-510) channel

For instrument specific guidance on correctly assigning Cq values follow manufacture instructions.

Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

Interpretation of Patient Specimen Results

If all the data analysis criteria are fulfilled, then each sample can be assessed with the following metric: Presumptive Positives:

		SARS-CoV-2 Target (FAM (465-510))	
		Cq Positive	Cq Negative
IEC (VIC / HEX / Yellow555 (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 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.

** The IEC Cq value produced by the patient sample should not exceed IEC Cq value + 6

produced by the NEC (sample RNA IEC Cq < NEC RNA IEC Cq + 6). Failure to satisfy this criterion indicates a compromised sample extraction and an invalid result; testing of the sample must be repeated.

16. Limitations of The Procedure

- The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay has been validated for use with oropharyngeal swab samples run on the Roche® LightCycler 480 II Real-Time PCR System, Bio-Rad CFX Connect™ Real-Time PCR Detection System, and Applied Biosystems® 7500 Real-Time PCR System with automated extraction system GenoXtract® from HAIN Lifescience GmbH (Brucker) using GXT DNA/RNA Extraction kit and with QIAamp® Viral RNA Mini kit (Qiagen, Germany).
- Nasal swabs and mid-turbinate nasal swabs are considered acceptable specimen types for use with the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
- 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 regions of the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- *In silico* analysis with Bat coronavirus (NCBI Accession No. MN996532.1) and Pangolin coronavirus (NCBI Accession No. MT084071.1) suggests that these two sequences may be detected with the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay.
- 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.
    - Use of unauthorized extraction kit or PCR platform.
- 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.

17. Conditions of Authorization for the Laboratory

The Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/ Emergency Situations/ucm161496.htm.

However, to assist clinical laboratories using the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and You (support@primerdesign.co.uk) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

[1] The letter of authorization refers to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

18. Performance Evaluation

The PrimerDesign Ltd COVID-19 genesig® Real-Time PCR assay performance evaluation has been generated on the Applied Biosystems® 7500 Real-Time PCR system with additional testing on the Roche® LightCycler 480 II and Bio-Rad CFX Connect™ Real-Time PCR Detection System instruments for analytical sensitivity (LoD).

18.1. Analytical Sensitivity

The Limit of detection (LoD) was defined as the lowest concentration of analyte that could be reliably detected at least 95% of the time. First a tentative LoD was detected by testing five limiting dilutions, prepared by spiking quantified SARS-CoV-2 whole viral RNA supplied by the European Virus Archive-Global (Cat: 026N-03889) into oropharyngeal swab sample negative for SARS-CoV-2. The LoD was confirmed by testing single replicates of the 20 extraction eluates on the three instruments: Applied Biosystems 7500 Real-Time PCR System, or Roche LightCycler 480 II, or Bio-Rad CFX 96 Connect Real-time PCR Detection System.
The LoD results are described in Sections 18.1.1 and 18.1.2.

18.1.1. Analytical Sensitivity Results

This data demonstrates that the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay detects 0.33 copies/µl of SARS-CoV-2 whole viral genome RNA ≥95% of the time. This concentration therefore is the limit of detection of the assay.

	ABI 7500 Real-Time PCR System (Applied Biosystems® 7500)					
	SARS-CoV-2Mean Concentration (copies/µl)	**Overall Mean Concentration (copies/rxn)**	Positive calls/Total no. results included in analysis	**% Replicate Detection**	**Mean Cq**	**Cq Standard Deviation**
	0.33	2.65	20/20	100	36.3	0.967

LoD of 0.33 copies/µl is maintained when using either GXT DNA/RNA Extraction kit or an alternative RNA extraction method, i.e. the QIAamp Viral RNA Mini kit.

18.1.2. Alternative Instrument Testing

The tentative LoD was also confirmed by testing 20 replicates on the CFX Connect™ Real-Time PCR Detection System (Bio-Rad) and LightCycler 480 II (Roche®) qPCR. The data confirmed that the LoD is 0.33 copies/µl.

	LightCycler 480 II (Roche®)	CFX96 (BioRad®)
SARS-CoV-2 Mean Concentration (copies/µl)	**% Replicate Detection**	
0.33	100% (20/20)	100% (20/20)

18.2. Inclusivity

In silico analysis showed that the primers/probe sequences of the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay have 100% homology with 52 SARS-CoV-2 sequences available on NCBI and 1,743 published good quality, full length sequences available on GISAID EpiCoV as of 27 March 2020.

18.3. Analytical Specificity

In silico analysis:
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 Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay exhibited no cross-reactivity with non-SARS-CoV-2 species except for the two sequences. Bat coronavirus (NCBI Accession No. MN996532.1) and Pangolin coronavirus (NCBI Accession No. MT084071.1) sequences, with 5 mismatches and 7 mismatches respectively and therefore show limited possibility of being detected with the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay.

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 by the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay 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

18.4. Clinical Performance Evaluation

Clinical evaluation of the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay was conducted with contrived oropharyngeal swabs (50 positive and 50 negative) in Copan universal transport medium. 50 swabs were contrived with SARS-CoV-2 whole viral genomic RNA (EVAg, Cat: 026N-03889) and tested blindly to generate the Positive Percentage Agreement (PPA) and Negative Percentage Agreement (NPA):

SARS-CoV-2 concentration	Results (Detected /Tested)	Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay % Positive (95% CIs)
1-2x LoD	36/38	94.7% (82.72 – 98.55)
3x LoD	7/7	100% (64.57 – 100)
4-5x LoD	5/5	100% (56.56 – 100)
Negative	50/50	100% (92.87 – 100)

19. Disposal

Dispose of unused kit reagents, human specimens and sealed post-amplification plates according to local, state and federal regulations.

20. Primerdesign Ltd Quality Control

In accordance with Primerdesign Ltd ISO 13485 certified Quality Management System, each batch of the Primerdesign Ltd COVID-19 genesig® Real-Time PCR 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.

21. Verification Requirements for Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay

21.1. SARS-CoV-2 RNA Verification Process

Purified SARS-Cov-2 whole viral RNA listed below is an example of a verification material. Laboratories can use other available appropriate materials to verify the Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay.

- SARS-CoV-2 whole viral RNA supplied by EVAg (Cat: 026N-03889) was used for this process. This SARS-CoV-2 RNA is purified from a cell culture of the Coronavirus strain "BetaCoV/Germany/BavPat1/2020 p.1".
- The estimated concentration of this material is 10^4 copies/µl. It should be opened and processed away from clinical specimens and kit components to avoid cross-contamination.
- Users are required to verify the SARS-Cov-2 whole viral RNA by preparing a four-point standard curve of three 1:10 serial dilutions down to 10 copies/µl:
    i) Label 3 x 1.5ml microcentrifuge tubes with the SARS-CoV-2 RNA concentration:
        - 10^3 copies/µl
        - 10^2 copies/µl
        - 10 copies/µl.
    ii) Pipette 90µl of RNase/DNase-free water into each labelled 1.5 ml microcentrifuge tubes.
    iii) Very briefly vortex and centrifuge SARS-CoV-2 RNA.
    iv) Perform a 1/10 dilution by transferring 10µl of the neat SARS-CoV-2 RNA into 10^3 copies/µl tube. Vortex briefly, change pipette tip.
    v) Perform a 1/10 dilution by transferring 10µl of 10^3 copies/µl tube into 10^2 copies/µl. Vortex briefly, change pipette tip.
    vi) Perform a 1/10 dilution by transferring 10µl of 10^2 copies/µl tube into 10 copies/µl. Vortex briefly.
- Prepare a reaction mix according to **Section 14.3**, ensure enough reaction mix is prepared for a single replicate of a positive control (at 1.7 copies/µl), a negative control and testing each dilution of the SARS-CoV-2 RNA in triplicate.
- Prepare a reaction plate according to **Section 14.3** and program your chosen PCR platform according to **Section 14.4.**
- Before interpreting the results, ensure that the following criteria is met:
    - NTC is free from amplification in the FAM (465-510) channel
    - PCT dilution at 1.7 copies/µl produces amplification in the FAM (465-510) channel
- All SARS-Cov-2 RNA reference material tested concentrations should produce amplification through the FAM channel.

22. Technical Support

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

Phone: +44 (0) 800 0156 494

Email: support@primerdesign.co.uk

23. Trademarks and Disclaimers

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

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

24. Explanation of Symbols

Symbol	Explanation
Rx	Prescription Use Only
IVD	In vitro diagnostics
	Manufacturer
REF	Catalogue number
	Suffices for
	Use by Date
	Temperature limit
	Consult Electronic Instructions for Use
LOT	Batch Code
	Keep away from sunlight (primer/probe mix)

Positive Control

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