# Xpert qDetect COVID-19

#GDK99.3500 (3x 500 qPCR rxns | ~500 samples)

(FOR RESEARCH ONLY)

**Product:**

**GRiSP´s Xpert qDetect COVID-19** is a qRT-PCR detection kit for **SARS-CoV-2**, based on real-time PCR assays for **N1**, **N2** and **RP**. The US Centers for Disease Control and Prevention (CDC) has developed and published a protocol for the detection of SARS-CoV-2 (2019-nCoV). This kit combines GRiSP´s highly efficient, fast and sensitive **Xpert One-Step Fast Probe** for qRT-PCR with the published primers and probes[1] from these CDC assays for the identification of this new coronavirus.

Viral sequences that have been targeted worldwide, so far, include ORF1ab, ORF1b-nsp14, E, S, three sequences in the N gene and two sequences in the RdRP gene[2]. **Be aware** that local guidelines for the detection and/or quantification of SARS-CoV-2 by qRT-PCR assays may be different those from CDC. Please check your government´s recommendations for the specific viral targets to be detected.

**This kit is meant for research only (RUO)**

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**Safety:**

In December 2019, a new betacoronavirus emerged in Wuhan, China and since then has spread throughout the world. The virus, initially referred to as 2019-nCoV, has now been named SARS-CoV-2 by the International Committee of Taxonomy of Viruses (ICTV). This virus can cause the disease named corona virus disease 2019 (COVID-19) and human-to-human transmission has been proven. Most frequent initial symptoms of COVID-19 that have been described include fever, cough, dyspnea, and bilateral infiltrates on chest imaging. In most reported cases, the course of infection is mild, yet, approximately 20% of confirmed patients have had critical illness (including respiratory failure, septic shock, or other organ failure requiring intensive care), of which a significant percentage resulted in a fatal outcome.

Therefore, ensure that adequate standard operating procedures (SOPs) are in use and that staff are trained for appropriate specimen collection, storage, packaging and transport. All specimens collected for laboratory investigation should be regarded as potentially infectious. Testing of clinical specimens from patients meeting the suspected case definition should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines and recommendations on laboratory safety should be followed in all circumstances. Specimen handling for molecular testing would require BSL-2 or equivalent facilities. Attempts to culture the virus require BSL-3 facilities at minimum.

**Before using** the Xpert qDetect COVID-19 qRT-PCR detection kit for the first time, GRiSP strongly recommends to adapt the World Health Organization´s guidelines (as well as National guidelines) regarding laboratory testing for COVID-19 [3] and Laboratory biosafety.[4]

# Index

## 1. Intended Use

GRiSP's Xpert qDetect COVID-19 is a qRT-PCR kit for the detection of SARS-CoV-2, based on real-time PCR assays for **N1**, **N2** and **RP**. It is for the use of COVID19 screening tests or the evaluation of anti-viral drug efficacy. Selected targets, corresponding primer sequences and probes are according to the protocol[1] published by the US CDC. Check your National government´s guidelines and recommendations to see which specific viral targets should be detected in your country, as they may be different. This product is for research only (RUO) and is to be used by trained laboratory professionals only.

## 2. Principle

GRiSP's Xpert qDetect COVID-19 is intended for the *in vitro* detection and quantification of SARS-CoV-2 RNA, previously isolated from human nasopharyngeal/tracheal/oropharyngeal apirates, swabs or washes, or sputum specimens (among others), in a one-step qRT-PCR reaction targeting 2 viral targets (**N1** and **N2**) using viral target specific primers and Taqman probe technology.

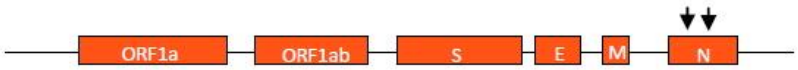



Figure 1. Schematic representation of the SARS-CoV-2 genome. ORF=open reading frame, S=Spike Protein gene. E=envelope protein gene, M=Membrane protein gene, and N=nucleocapsid protein. The gene encoding the RNA-dependent RNA polymerase (RdRp) is located on ORF1ab. The arrows indicate the viral targets of the Xpert Detect COVID-19 kit.

The human RNase P gene (**RP**) is used both as internal and extraction control to evaluate viral RNA extraction efficiency as well as to control qRT-PCR performance. Primer and Probe sequences can be found in the appendix. Primers and probes have been optimized to run under the same cycling conditions, thus analysis for N1, N2 and RP can be done in a single run (see also section 9: recommended pipetting scheme). All probes are FAM-labeled; hence this kit is to be used with real-time PCR instruments equipped with a FAM-channel.

## 3. Kit Content

The qPCR Detection Kit (#GDK99.3500) for SARS-CoV-2 contains sufficient reagents to for 500 qPCR reactions for each N1, N2 and RP (1500 rxns in total), allowing to test ~500 samples.

| | Product | GDK99.3100 | |
|---|---|---|---|
| | PP Mix N1 | 1x | 750 µl |
| | PP Mix N2 | 1x | 750 µl |
| | PP Mix RP | 1x | 750 µl |
| | Fast qPCR Mastermix (Probe) | 15x | 1ml |
| | RTase Mix | 15x | 100 µl |
| | 100x ROX (50µM) reference dye | 5x | 200 µl |
| | PCR-grade Water (RNase-free) | 5x | 1ml |
| | Positive Control (P) | 1x | 400 µl |
| | Human Specimen Control (HSC) | 1x | 100 µl |

## 4. Storage Conditions

Depending on the estimated delivery time, Xpert qDetect COVID-19 is shipped at room temperature or cooled with blue ice packs. Upon receipt, all individual components should be stored at -15ºC to -30ºC, and protected from light. All components are stable for at least 1 year. Minimize repeated freeze/thawing (consider preparation of aliquots).

## 5. Other Required Materials and Devices (not provided with this kit)

*Reagents and Devices for viral RNA isolation:*
The quality and quantity of the isolated viral RNA is crucial for the detection and quantification accuracy. GRiSP's Xpert qDetect COVID-19 is compatible with GRiSP's GK12.0100 GRS Viral DNA/RNA Purification Kit as well as with a large variety of other commercial available kits for the isolation of Viral RNA.

*Consumables:*
Nuclease-Free pipette tips with aerosol barriers, Nuclease-Free microcentrifuge tubes, and Nuclease-Free PCR tubes or plates and seals that are compatible with your specific qPCR-platform.

*Equipment*:
qPCR instrument, dedicated pipettes for each step of the protocol (sample preparation, qRT-PCR mastermix preparation, sample transferring to PCR tubes or plates, etc). Microcentrifuge, Benchtop centrifuge for PCR tubes, strips or plates, Spectrophotometer.

## 6. General Precautions

- Ensure that adequate standard operating procedures (SOPs) are in use
- Ensure that staff are trained for appropriate specimen collection, storage, packaging and transport
- Ensure National guidelines and recommendations on laboratory safety are followed in all circumstances
- All specimens collected for laboratory investigation should be regarded as potentially infectious
- The positive control is a plasmid harboring the complete nucleocapsid gene (N) from SARS-CoV-2. For safety, it should be treated as infectious material
- Wear appropriate personal protective clothing, including gowns, disposable gloves and eye protection, throughout the assay procedure.
- Specimen processing should be carried out in a certified BSL-2 laboratory following biosafety level 2 guidelines or higher.
- Latex gloves must be changed and disposed of before leaving the area.
- Use disposable plasticware
- Automatic pipettes and non-disposable glassware or plasticware should be nuclease-free
- Use dedicated pipettes for each individual step in the procedure.
- Use pipette tips with aerosol barriers
- Thaw all components thoroughly at room temperature, mix the components and centrifuge briefly before starting an assay. Avoid repeated freeze/thaw cycles.
- Thoroughly clean and disinfect, periodically, all work surface and devices (e.g. with 5% sodium hypochlorite, followed up by drying with 70% ethanol).
- To avoid cross-contamination, workflow in the laboratory must be in a uni-directional manner from sample collection area to RNA isolation area to Pre-amplification area to Post-Amplification Area.
- Use isolated RNA for immediately after extraction or store at -20ºC (short time)/-70ºC.
- Before disposal, all waste materials should be autoclaved at 121.1ºC for at least one hour or incinerated. Dispose of all waste materials according to National Legislation.

## 7. Specimen preparation and RNA isolation

Specimen handling, preparation and storage should be carried out according to National guidelines or recommendations. SARS-CoV-2 RNA should be isolated from specimen using a specific commercial kit for the isolation of viral RNA (e.g. GRiSP´s GK12.0100 GRS Viral DNA/RNA Purification Kit) using the manufacturer´s instructions. Extracted RNA should be used immediately, or stored for a very short time at -20ºC or at -70ºC or lower, as soon as possible. It is recommended to check RNA concentration and integrity. A260nm/A280nm should ~2.0

## 8. Controls

Each assay run must include the following controls:

- SARS-CoV-2 Positive Control (**P**)
  This is a plasmid harboring the complete nucleocapsid gene (N) from SARS-CoV-2. This is not a RNA control. It has been included in this kit for your convenience, in case a viral RNA control is not obtainable. Positive controls must show up positive for both targets N1 and N2 and negative for RP for the run to be valid

- No Template Control (**NTC**)
  Nuclease-Free water should be used as NTC and must show up negative for all targets N1, N2 and RP, assuring the absence of cross-contamination of reagents

- Extraction Control (**EC**)
  An Extraction Control is obtained by isolating RNA from a virus-free specimen. The EC will serve as the negative control for the whole procedure as well as an internal control (IC) for the RTase activity.

In case no amplification for RP is observed in the extraction control (EC) or in none of the samples, run a test using the HSC:

- Human Specimen Control (**HSC**)
  This is a plasmid harboring a portion of the human RPP30 gene that encodes a subunit of RNase P. This is not a RNA control. It has been included in this kit for your convenience, in case a RNA control is not obtainable. This control should be used if samples are frequently negative in RP (**see section 14: Troubleshooting**)

## 9. Equipment and ROX

Unambiguous detection of SARS-CoV-2 requires a suitable calibration/validation of the FAM-channel. Please refer to the manufacturer´s instruction of the real time PCR cycler.

Appropriate reaction plates with optical closing seals or reaction tubes with optical closing lids should be used. Please refer to the manufacturer´s instruction of the real time PCR cycler.

The passive reference dye **ROX** compensates for variations in fluorescence detection that are unrelated to the PCR reaction. The fluorescence level of ROX provides a stable baseline during cycling against which PCR-related fluorescence signals are normalized. Thus, differences between samples due to variations in reaction volumes caused by pipetting are adjusted. As the dye does not inhibit the PCR reaction and has a completely different emission spectrum, it does not interfere with qPCR on any equipment.

Some equipment require no ROX, whereas others require low ROX or high ROX. Please refer to the manufacturer´s instructions of the real time PCR cycler. Set-up instruments software accordingly. If you are not sure whether your equipment requires ROX or how much, or if you simply do not want to use the benefit of ROX, as it is not essential, you can perform reactions without ROX. Make sure to uncheck ROX in your instrument´s software, if applicable. Please refer to the manufacturer´s instruction of the real time PCR cycler.

## 10. Preparation of Assay Mixes

Before starting, determine the required volume of each of the individual components, based on the amount of samples to be tested, including all controls and some excess to compensate for pipetting errors. Then, in order to minimize repeated freeze-thaw cycling, remove from the freezer only the required amount of tubes. Do not leave unused kit components at room temperature for more than 2 hours.

Thaw all components thoroughly at room temperature for a minimum of 30 minutes. Mix all components by inverting several times and centrifuge briefly before use. When handling, minimize exposure to direct light, as exposure for an extended period of time might result in the loss of signal intensity.

### 10.1 Preparation of Fast qPCR Mastermix (Probe)

Depending on your equipment (**see section 9**) prior to use for the first time, add 20µl (in case of "High ROX" or 2µl (in case of "Low ROX") of the 100x ROX reference dye to each 1-ml vial of the **Fast qPCR Mastermix (Probe)** and mix by inverting the vial several times. If your instrument is "No ROX" (or if you prefer not to use ROX) then you should use the Fast qPCR Mastermix (Probe) as is, thus without adding ROX. After adding ROX, the mastermix can be used directly or stored at -20ºC for up to 1 year.

### 10.2 Preparation of Combined Primers/Probe Mixes

Combined Primer/Probe Mixes (PP Mix) for each of the three targets (N1, N2 and RP) are already prepared and thus ready-to-use. Do not alter their composition nor dilute. Concentrations of primers and probes have been optimized and details regarding sequences and concentrations can be found in the appendices.

### 10.3 Preparation of qRT-PCR Reaction Mixes

The amounts described in this section, are based on the analysis of a 5µl viral RNA sample. This means that a total of 15µl viral RNA sample is required, as each sample must be tested for N1, N2 and RP. If less viral RNA sample is available, add PCR-grade Water (RNase-free) to the viral RNA sample to ensure final reaction volumes are 20µl (15µl of reaction mix and 5µl of sample).

| Component | Volume |
|---|---|
| Fast qPCR Mastermix (Probe) with/without ROX[1)] | 10 µl |
| RTase Mix | 0.5 µl |
| PP Mix N1 **or** PP Mix N2 **or** PP Mix RP | 1.5 µl |
| PCR –grade water (RNase-free) | 3.0 µl |

[1] ROX as required, see sections 9 and 10.1
[2] The mixtures have already been optimized. It is not recommended to add further components..

For each sample to be tested, prepare 3 separated qRT-PCR Reaction Mixes, one for each N1, N2 and RP. Label tubes accordingly. In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare qRT-PCR Reaction Mixes for multiple samples, always including a positive control (P), an extraction control (EC) and a No Template Control (NTC) (**see section 8**) and some excess to compensate for pipetting errors (5-10%).

For example, when preparing a qRT-PCR Reaction Mix for the analysis of 29 samples and 3 controls (which corresponds to a full 96-well PCR plate according to the suggested plate arrangement in **section 11**), prepare **N1 Mix** for by mixing 350µl (35x 10µl) of Fast qPCR Mastermix (Probe (with or without ROX) and 17.5µl (35x 0.50µl) of RTase Mix, 52.5µl (35x 1.5µl) of PP Mix N1, and 105µl (35x 3µl) of PCR-grade water (RNase-free) in a RNase/DNase-free microcentrifuge tube.
Prepare **N2 Mix** using PP Mix N2 and **RP Mix** using PP Mix RP, in similar way.

## 11. Suggested Plate Arrangement

Hereunder you can find a suggested plate arrangement for processing 29 samples in a single experiment using a 96-well PCR plate. Start by adding 15µl of the corresponding qRT-PCR Reaction Mix (N1 Mix, N2 Mix, RP Mix) and then add 5µl of template.

| | N1 | N2 | RP | N1 | N2 | RP | N1 | N2 | RP | N1 | N2 | RP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | **NTC** | **NTC** | **NTC** | S8 | S8 | S8 | S16 | S16 | S16 | S24 | S24 | S24 |
| B | S1 | S1 | S1 | S9 | S9 | S9 | S17 | S17 | S17 | S25 | S25 | S25 |
| C | S2 | S2 | S2 | S10 | S10 | S10 | S18 | S18 | S18 | S26 | S26 | S26 |
| D | S3 | S3 | S3 | S11 | S11 | S11 | S19 | S19 | S19 | S27 | S27 | S27 |
| E | S4 | S4 | S4 | S12 | S12 | S12 | S20 | S20 | S20 | S28 | S28 | S28 |
| F | S5 | S5 | S5 | S13 | S13 | S13 | S21 | S21 | S21 | S29 | S29 | S29 |
| G | S6 | S6 | S6 | S14 | S14 | S14 | S22 | S22 | S22 | **EC** | **EC** | **EC** |
| H | S7 | S7 | S7 | S15 | S15 | S15 | S23 | S23 | S23 | **P** | **P** | **P** |

P= Positive Control, EC = Extraction Control, NTC = No Template Control (**section 8**)
S1-S29: samples 1 to 29. Other run layouts are also possible, as long as each plate includes at least one NTC, one EC and one P control for each of the targets tested.

After adding all reagents and samples to the wells, tightly seal the plate to prevent evaporation. Centrifuge at 1000 rpm and place in the real time PCR instrument immediately.

## 12. Reaction Set-up

Set-up the qRT-PCR reaction conditions as outlined in the table below on calibrated/validated instruments, according to manufacturer´s instructions. Select FAM channel for data acquisition for all samples and controls.

| Step | Nº cycles | Temp | Time | Data acquisition |
|---|---|---|---|---|
| cDNA synthesis | 1x | 45ºC | 15 min | NO |
| Enzyme activation (hot start) | 1x | 95ºC | 2 min | NO |
| 2-step qPCR cycling | 45x | 95ºC | 15 sec | NO |
| | | 55ºC | 30 sec | FAM |

## 13. Data Analysis

After the run has been completed, save and analyze the data following the instrument manufacturer´s instructions. Please check amplification curves. In case curves do not have standard shape but are too much of an S-shape, auto baseline subtractions may have set the end cycle too low. Adjust by setting baseline manually. Verify visually for each sample, whether the threshold was set correctly. The threshold should be set to above any background signal to around the middle of the exponential (geometric) phase of the amplification curve in the logarithmic view. The procedure for setting the threshold should be consistent between runs. Samples should be inspected both in logarithmic and linear scale views and compared with the controls.

### 13.1 Evaluation of the Assay Run

In order for the run to be valid, it must meet all the following criteria:

- No amplification is observed for the No Template Control (NTC) in each of the three reaction mixes (N1 Mix, N2 Mix and RP Mix). Cq should be undetermined
- No amplification is observed for the Extraction Control (EC) in each of the two reaction mixes (N1 Mix and N2 Mix), and amplification is observed in RP Mix (Ct<32)
- Amplification should be observed for the positive control (P) in both N1 and N2 mix (Ct<37), and no amplification is observed in RP Mix.

| control | N1 Mix | N2 Mix | RP Mix |
|---|---|---|---|
| NTC | negative | negative | negative |
| EC | negative | negative | positive |
| P | positive | positive | negative |

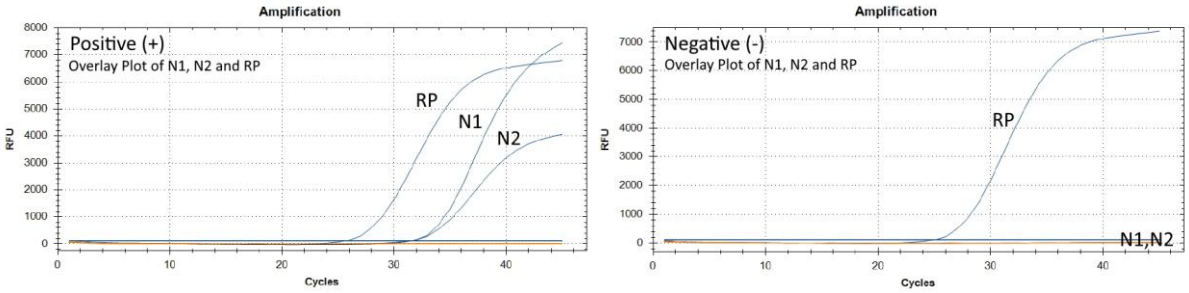
If not all criteria are met, all results should be discarded and the tests must be repeated (see also: **section 14 Troubleshooting**)

### 13.2 Evaluation of Samples

Upon positive evaluation of the Assay Run (**section 13.1**) assess the results of each individual assay based on the obtained Ct values as outlined in the table below. It is recommended to visually check every single curve to verify if threshold was set correctly and whether amplification curve has a standard shape.

| N1 Mix | N2 Mix | RP Mix | COVID-19 result |
|---|---|---|---|
| Negative (Ct>40) | Negative (Ct>40) | Positive (Ct<32) | negative |
| Positive (Ct<38) | Positive (Ct<38) | Any value | positive |
| Negative (Ct>40) | Negative (Ct>40) | Negative (Ct>32) | invalid sample (re-isolate viral RNA and repeat the test) |
| Positive (Ct<38) | Negative (Ct>40) | Any value | Inconclusive, repeat the test |
| Negative (Ct>40) | Positive (Ct<38) | Any value | Inconclusive, repeat the test |
| Inconclusive 38≤Ct<40 | Any value | Any value | Inconclusive, repeat the test |
| Any value | Inconclusive 38≤Ct<40 | Any value | Inconclusive, repeat the test |

It may be possible that a sample tests positive for N1 and negative/inconclusive for N2 (or vice versa). This may be due to the fact that viral sample concentration is near or below the limit of detection or alternatively due to a mutation in the corresponding target region.




### 13.3 Quantification

For accurate quantification, run a standard curve, using well defined template RNA, to determine the limit of detection and the correlation between concentration and Ct. At lower concentration, the amplification curves may begin to group together and consequently Ct values will no longer fit the standard curve. In order to determine target concentration in given sample, Ct value must fit the linear range. In order to correct for well to well inconsistencies, for precise quantification, normalization by using ROX is required (depending on the instrument, another passive dye maybe required, please refer to manufacturer´s instructions).

## 14. Troubleshooting

| Problem | Possible Cause(s) | Solution(s) |
|---|---|---|
| Fluorescence signals in NTC for N1 or N2 or RP and/or in the EC for N1 or N2 | Contamination during PCR set-up | Repeat the qRT-PCR with new reagents. Follow GLP in the PCR Lab. Workflow in the laboratory must be in a uni-directional manner from sample collection area to RNA isolation area to Pre-amplification area to Post-Amplification Area. Decontaminate workspace and instruments regularly, |
| | Signal doesn't correspond to target amplification but to background curves | Ignore the Ct value of NTC in case the aspect of the amplification curve is abnormal and resembles background noise |
| No Fluorescence signals in P for N1 or N2 | Incorrect programming of the real-time PCR instrument | Compare the temperature profile with the protocol (section 12) |
| | Incorrect preparation of Mastermixes | Repeat the test with newly prepared mixes. Check info about ROX reference dye and instrument/software set-up |
| No Fluorescence signals in samples in RP (nor N1, N2) | If in just a few samples | If most samples, but not all, give a positive signal for RP, it is very likely that RNA isolation of the samples that did not amplify was done incorrectly or RNA was stored inadequately and degraded. Repeat Viral RNA isolation and repeat the test |
| | If in almost all samples | If almost all samples give a negative signal for RP, Either Viral RNA isolation was done incorrectly for all those samples or RTase may have been compromised. Check RTase and qPCR by running a well-defined EC along the HSC, both in triplicate against RP. If HSC is positive but EC not, it is likely RTase was not functional. Compare the temperature with the procotol (secion 12) and repeat with new tube of RTase. |

## 15. References

1. CDC/DDID/NCIRD (2020) 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, Instructions for use. CDC-006-00019, Revision 3 Effective of 30-03-2020 (url: https://www.fda.gov/media/134922/download )
2. WHO (2020) Shared "in-house" protocols at the WHO website at the beginning of the COVID-19 outbreak (url: https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2 )
3. WHO (2020) Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. Interim guidance 19 March 2020 (url:https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117)
4. WHO (2020) Laboratory biosafety guidance related to coronavirus disease (COVID-19. Interim guidance 19 March 2020 (url: who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19))

## Appendices

### A1. Primers and Probes

| Name | Description[1] | Sequence | Conc (PP Mix) | Conc (final) |
|---|---|---|---|---|
| N1 Forward primer | 2019-nCoV_N1-F2019-nCoV_N1 | GACCCCAAAATCAGCGAAAT | 6.7µM | 500nM |
| N1 Reverse primer | 2019-nCoV_N1-R2019-nCoV_N1 | TCTGGTTACTGCCAGTTGAATCTG | 6.7µM | 500nM |
| N1 Probe | 2019-nCoV_N1-P2019-nCoV_N1 | **FAM**-ACCCCGCATTACGTTTGGTGGACC | 1.7µM | 125nM |
| N2 Forward primer | 2019-nCoV_N2-F2019-nCoV_N2 | TTACAAACATTGGCCGCAAA | 6.7µM | 500nM |
| N2 Reverse primer | 2019-nCoV_N2-R2019-nCoV_N2 | GCGCGACATTCCGAAGAA | 6.7µM | 500nM |
| N2 Probe | 2019-nCoV_N2-P2019-nCoV_N2 | **FAM**-ACAATTTGCCCCCAGCGCTTCAG | 1.7µM | 125nM |
| RP Forward primer | RP-F_RNAseP_Forward | AGATTTGGACCTGCGAGCG | 6.7µM | 500nM |
| RP Reverse primer | RP-R_RNAseP_Reverse | GAGCGGCTGTCTCCACAAGT | 6.7µM | 500nM |
| RP Probe | RP-P_RNAseP_Probe | **FAM**-TTCTGACCTGAAGGCTCTGCGCG | 1.7µM | 125nM |

*The CDC has quality controlled and authorized primers and probes from batches included in this kit for the N1, N2 and RP assays. Probes are 5´-FAM labeled and 3´-Black Hole Quencher® labeled.*

### A2 Controls

**A2.1** SARS-CoV-2 Positive Control (2019-nCoV_N_Positive Control) **(P)**

This is a plasmid harboring the complete nucleocapsid gene (N) from SARS-CoV-2. The complete sequence is shown hereunder. Highlighted in blue are the binding sites for N1 primers and probe and highlighted in yellow are the binding sites for N2 primers and probe. It is supplied at a concentration of 200 copies/µl.

*Disclaimer:* This positive control (P) is not a RNA control, but a DNA control. It has not been designed, tested, or validated by CDC. This control is offered as a convenience to the end-user, as a viral RNA control is often not obtainable, and should be validated by the end-user if used for the interpretation of test results.

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCG
GATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCG
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ACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGC
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AATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCT
CGTGCGCTCTCCTGTTCCGACCCTGTCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCT
CATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTT
CAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACT
ACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC
TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAA
AGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTT
GGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATA
TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCAT
CCATAGTTGC

**A2.2** RP positive control (Hs-RPP30_Positive Control) **(HSC)**

This is a plasmid harboring a portion of the RPP30 gene, a single copy housekeeping gene present in the human genome that encodes a subunit of RNase P (RP). Highlighted in purple are the binding sites for RP primers and probe. It is supplied at a concentration of 200 copies/µl.

*Disclaimer:* This positive control (HCS) is not a RNA control, but a DNA control. It has not been designed, tested, or validated by CDC. This control is offered as a convenience to the end-user, as a RNA control is often not obtainable, and should be validated by the end-user if used for the interpretation of test results.

CCCGTGTAAAACGACGGCCAGTTTATCTAGTCAGCTTGATTCTAGCTGATCGTGGACCGGAA
GGTGAGCCAGTGAGTTGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCTGAATGAT
ATGCGGCCTCGGACTTCAGCATGGCGGTGTTTGCAGATTTGGACCTGCGAGCGGGTTCTGA
CCTGAAGGCTCTGCGCGGACTTGTGGAGACAGCCGCTCACCGTGAGTTGCGGTCTCCCAGA
GTCTCTGGGATGTCCCTGGAGGCTGATGGCGCGTGATCTTACGGCATTATACGTATGATCGG
TCCACGATCAGCTAGATTATCTAGTCAGCTTGATGTCATAGCTGTTTCCTGAGGCTCAATACT
GACCATTTAAATCATACCTGACCTCCATAGCAGAAAGTCAAAAGCCTCCGACCGGAGGCTTT
TGACTTGATCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGG
CGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGAGTATTCAACATTTC
CGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACG
CTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGA
TCTCAACAGCGGTAAGATCCTTGAGAGTTTACGCCCCGAAGAACGTTTTCCAATGATGAGCA
CTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTC
GGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCA
TCTCACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACA
CTGCGGCCAACTTACTTCTGGCAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCAC
AACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACC
AAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAA
CTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAA
GTTGCAGGATCACTTCTGCGCTCGGCCCTCCCGGCTGGCTGGTTTATTGCTGATAAATCTGG
AGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC
CGCATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGAT
CGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAATGAGGGCCCAAATGTAATCACCTGG
CTCACCTTCGGGTGGGCCTTTCTTGAGGACCTAAATGTAATCACCTGGCTCACCTTCGGGTG
GGCCTTTCTGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAA
TCGATGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCC
CCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCG
CCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCG
GTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT
GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTG
GCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCT
TGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTG
AAGCCAGTTACCTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGT
AG