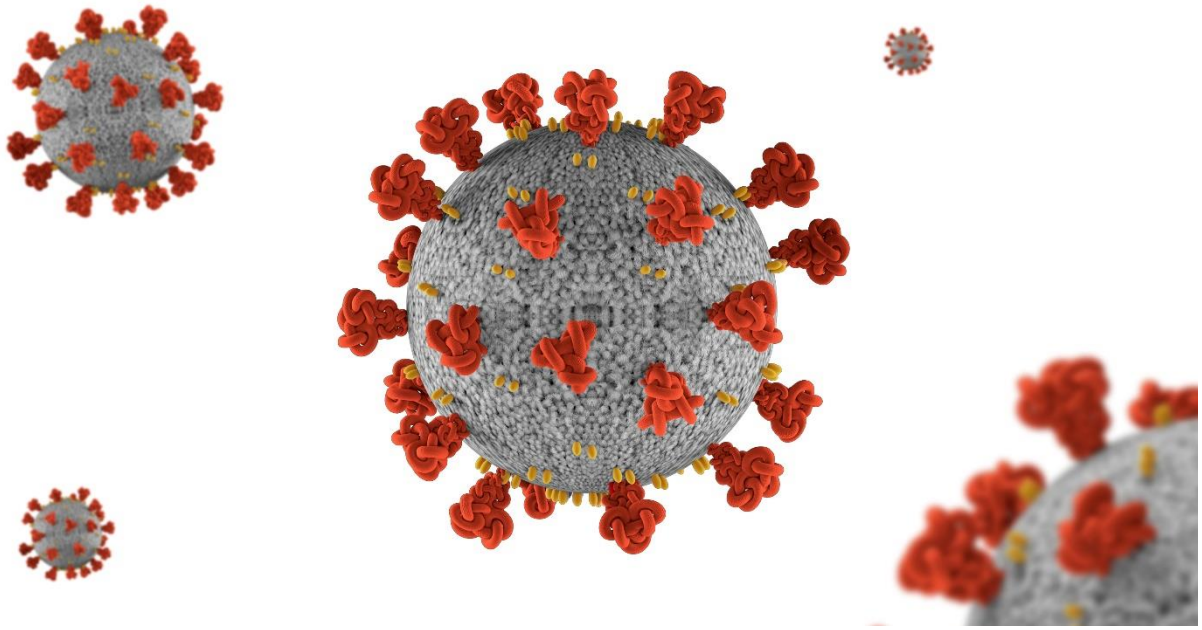
Real Time RT-PCR primers and probes for SARS-CoV-2 *in vitro* detection



Version 1.1

Product description:

This document describes the use of real-time RT PCR (rRT-PCR) assays for the *in vitro* detection of 2019-Novel Coronavirues (2019-nCoV) in respiratory specimens and sera, e.g. nasopharyngeal aspirates or washes, swabs, bronchioalveolar lavage and sputum.

Prepare RNA specimens using RNA extraction Kit or manual method for RNA extraction.

Biosafety precautions

Wear appropriate personal protective equipment (e.g. gloves, eye protection) when working with clinical specimens. Specimens processing should be performed in an appropriated certified biological safety laboratory environment.

Nucleic Acid Extraction

The quality of the assay is largely dependent on the quality of input RNA. RNA extraction procedures should be qualified and validated for recovery and purity before testing specimens.
Commercially available extraction procedures that have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction include:

-QIAamp® Viral RNA Mini Kit,

-QIAamp® MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN),

-EZ1 DSP Virus Kit (QIAGEN),

-Roche MagNA Pure Compact RNA Isolation Kit,

-Roche MagNA Pure Compact Nucleic Acid Isolation Kit,

-Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit,

-Invitrogen ChargeSwitch® Total RNA Cell Kit.

Specimens can be stored at 2-4 °C for up to 72 hours after collection. If the sample was not kept at 2-4°C for up to 72 hours after collection it should be considered not valid for the test.
Only thaw the number of specimen extracts that will be tested in a single day. Do not freeze/thaw extracts more than once before testing.

Important Note: This document has been prepared based on the TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher; cat # A15299 or A15300). If a different system will be used please adjust it based on the supplier instructions.

Information in this document is subject to change without notice. baseclick GmbH assumes no responsibility for any errors that may appear in this document.

baseclick GmbH disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall baseclick GmbH be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

Please read the material safety data sheets (MSDS) provided for each product/component.

1. Primers and probes

The primers and probes sets are designed for the universal detection of SARS-like coronaviruses (N3 assay) and for the specific detection of 2019-nCoV (N1 and N2 assays).

RP - all clinical samples should be tested for human RNAse P (RNP) gene to assess specimen quality

Name	Sequence (5'-3')	Lenght	Label.	Working Conc.
BCC-19nCoV_N1 Forward Primer	GAC CCC AAA ATC AGC GAA AT	20	no	20 µM
BCC-19nCoV_N1 Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG	24	no	20 µM
BCC-19nCoV _N1 Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1	24	FAM / BHQ1	5 µM
BCC-19nCoV _N2 Forward Primer	TTA CAA ACA TTG GCC GCA AA	20	no	20 µM
BCC-19nCoV _N2 Reverse Primer	GCG CGA CAT TCC GAA GAA	18	no	20 µM
BCC-19nCoV _N2 Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG -BHQ1	23	FAM / BHQ1	5 µM
BCC-19nCoV _N3 Forward Primer	GGG AGC CTT GAA TAC ACC AAA A	22	no	20 µM
BCC-19nCoV _N3 Reverse Primer	TGT AGC ACG ATT GCA GCA TTG	21	no	20 µM
BCC-19nCoV _N3 Probe	FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1	24	FAM / BHQ1	5 µM
BCC-19nCoV RNAse P Forward Primer	AGA TTT GGA CCT GCG AGC G	19	no	20 µM
BCC-19nCoV RNAse P Reverse Primer	GAG CGG CTG TCT CCA CAA GT	20	no	20 µM
BCC-19nCoV RNAse P Probe	FAM- TTC TGA CCT GAA GGC TCT GCG CG - BHQ1	23	FAM / BHQ1	5 µM

2. Required Material and Equipment not included in this Kit

- Positive template control
- TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher; cat # A15299 or A15300)
- Molecular grade water, nuclease-free
- Sterile, nuclease-free 1.5 mL microcentrifuge tubes
- 0.2 mL PCR reaction tube strips or 96-well real-time PCR reaction plates and optical 8-cap strips
- Acceptable surface decontaminants e.g. DNA AwayTM (Fisher Scientific; cat. #21-236-28) or RNAse AwayTM (Fisher Scientific; cat. #21-236-21 or 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)
- PCR Work Station [UV lamp; Laminar flow (Class 100 HEPA filtered)]
- Vortex mixer
- Microcentrifuge
- Real-time PCR detection system
- Nucleic acid extraction system (as described above)

3. Reaction set-up

The performance of the PCR step must be done on a real-time quantitative PCR system. Traditional thermal cyclers cannot detect and record fluorescent signal data generated by the cleavage of hydrolysis probes

Instrument settings: Detector (FAM; FITC); Quencher (None); Passive Reference: (None); Run Mode: (Standard); Sample Volume (20 µL)

NOTE: This protocol is suggested as a starting point. Optimal reaction conditions—incubation times and temperatures, primer/probe concentration, and the amount of template—can vary and should be optimized.

Step	Cycles	Temperature	Time
UNG incubation	1	25 °C	2 minutes
RT incubation	1	50 °C	15 minutes
Enzyme activation	1	95 °C	2 minutes
AMplification	45	95 °C	3 second
		55 °C*	30 seconds

* Fluorescence data (FAM) should be collected during the 55 °C incubation step.

Prepare RT-qPCR reaction mix

- Thaw all reagents on ice
- Vortex briefly to mix, then centrifuge to collect

Componet	Volume	Notes
4X TaqPath™ 1-Step RT-qPCR Master Mix	5 µL	n.r.
User-defined assay (primer and probes)	1 µL	Recommended primer concentrations of 400–900 nM and a probe concentration of 100–250 nM*
Sample	Variable (100ng to 1 pg)	Use as much sample as needed, up to the maximum allowed by the reaction volume
RT-qPCR Grade Water	Up to 20 µL	n.r.

*Refer to the rRT-PCR kit supplier

- Working on ice, add the components directly to each well of an optical reaction plate.
- Cover the reaction plate with an optical adhesive cover, invert the plate 3–5 times, making sure that the contents of the wells are moving back and forth between the seal and the bottom of the wells to ensure proper mixing, then centrifuge at 150 × g for 1 minute to spin down the contents and eliminate air bubbles.

4. Run the rRT-PCR and analyze the data

Notes:

For References and FAQs see online

To place your order, please contact us under:

phone: +49 89 9699 3401

fax: +49 89 9699 4696

online: www.baseclick.eu

email: orders@baseclick.eu