Efficient 2019-nCoV Detection Kit – 1-Step RT-qPCR User Guide

1-Step RT-qPCR kit (with N1, N2, RP primer & Probe Sets)
for detection of 2019-nCoV - 100 RT-qPCR reactions
Cat # 19nCoVd-100

For Research Use Only
Store at -20°C & keep away from light

I. Product information

1) Introduction

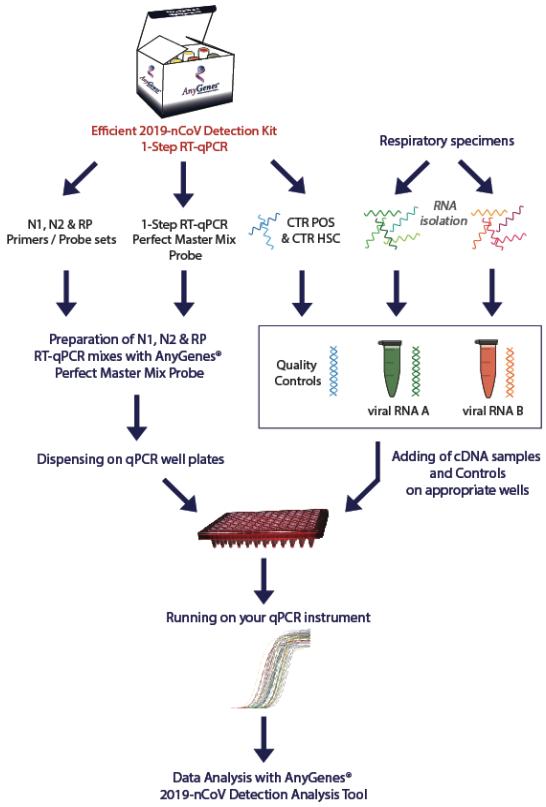
This kit has been developed following instructions from the Emergency Use Authorisation from the FDA. It also includes the recommended FAM-BHQ probes (Black Hole Quencher®).

✓ **Principles of the procedure**

The AnyGenes® Efficient 2019-nCoV Detection Kit is a ready-to-use system for the detection of novel coronavirus (2019-nCoV) RNA by 1-Step RT-qPCR technology.
This kit contains:

- Primers and probe sets for detection of 2019-nCoV, specific to regions of the virus nucleocapsid (N) gene (2 different sites) and human RNase P gene (RP) as control, according to CDC recommendations (Centers for Disease Control and Prevention)
- RT-qPCR Perfect Master Mix Probe, especially developed and optimized for this application
- Additional quality controls (CTR POS for positive quality control and CTR HSC as quality control of RNA isolation) for each sample and qPCR experiment.

The performance of AnyGenes® Efficient 2019-nCoV Detection Kit has been carefully optimized with our own 1-Step RT-qPCR Perfect Master Mix Probe kit to provide you a high sensitivity and specificity.
For details see **www.anygenes.com**.



✓ **Quality Control**

As part of our routine quality assurance program, all AnyGenes® products are monitored to ensure the highest levels of performance and reliability.

2) Intended use

This Efficient 2019-nCoV Detection Kit is a RT-qPCR panel for the qualitative detection and amplification of nucleic acids from new coronavirus 2019-nCoV. These kits are only developed as **Research Use Only (RUO)** applications.

3) Kit contents

This Efficient 2019-nCoV Detection Kit (for 100 RT-qPCR reactions) contains :

Catalog Ref	Contents
19nCoVd-100	**Efficient Reverse Transcriptase** vial (200 µl) **Efficient 1-Step RT-qPCR Perfect Master Mix Probe** vial (1 mL) **VPP-N1-100** vial (10X N1 Viral Primers & Probe set / 200 µl) **VPP-N2-100** vial (10X N2 Viral Primers & Probe set / 200 µl) **VPP-RP-100** vial (10X RP Primers & Probe set / 200 µl) **CTR-POS-100** vial (4X CTR POS / 600 µl) **CTR-HSC-100** vial (4X CTR HSC / 600 µl) PCR grade H2O vial (1 ml)

For more product information, please visit **www.anygenes.com** or contact us at **technical@anygenes.com**

4) Storage & stability

Upon receipt, store Efficient 2019-nCoV Detection Kit at -20°C until their use. These storage conditions guarantee a long-term storage of AnyGenes® products for a minimum period of 6 months after their receipt. Moreover, in order to guarantee the stability of these products, avoid repeated freezing and thawing cycles. If small volumes of this kit are frequently required, we recommend to stock alicots at -20°C.

5) Additional reagents and equipment required

A) Reagents :

- RNA isolated from respiratory specimens
- PCR grade H2O (supplied with AnyGenes® Efficient 2019-nCoV Detection Kit)

Caution: Do not use DEPC H_2O !!!

B) Material :

- Real-time quantitative PCR instrument (LightCycler® 480 (Roche®), ABI 7900®, ABI 7500® (Applied Biosystems® / Life Technologies®)...)
- qPCR plate or SignArrays® 96- or 384-well plates
- PCR plates centrifuge
- Vortex mixer and Mini-centrifuge
- "nuclease, RNase, DNase free" tips and tubes
- Pipettes for reaction mix preparation and dispending

II. Protocol

1) Before you start...

To obtain reliable and reproducible results and avoid contamination and false-positive signals, it is very important and necessary to follow Good Laboratory Practices.
You will be in contact and exposed with specimens containing viral material, to be tested with our qPCR reagents and panels. Please strictly respect and follow the recommendations establish in your lab and read carefully this protocol before performing any experiment.

✓ **Sample Preparation**

Please perform previous RNA isolation with appropriate kits for respiratory specimens, by ensuring of the good quality of the isolated RNA.

2) Procedure

1- Thaw AnyGenes® Efficient 2019-nCoV Detection Kit, 20 minutes before use, in order that slowly reaches 2-8°C temperature, on ice.

2- Prepare the work area (under workstation and appropriate equipment) by carefully cleaning all material and areas with a suitable detergent and then decontaminating the workstation through exposure to UV.

3- Meanwhile, briefly centrifuge tubes and reagents and prepare the RT-qPCR reaction mix **for each Primers & Probe set** (N1, N2 and RP) in a 1.5 ml tube according to the following table:

Reagents	Volumes / reaction	Volumes x n reactions
Efficient Reverse Transcriptase	2 µl	n x 2 µl
Efficient 1-Step RT-qPCR Perfect Master Mix Probe	10 µl	n x 10 µl
Primers & Probe set (N1, N2 or RP)	2 µl	n x 2 µl
Total Reaction Volume	**14 µl**	**n x 14 µl**

4- Mix thoroughly with a pipette and briefly centrifuge each RT-qPCR mix.

5- Dispense **14 µl** per well of **appropriate RT-qPCR reaction mix** on the qPCR plate. Then add **6 µl** per well **of RNA, corresponding quality control** (CTR POS or CTR HSC), **or PCR grade H_2O** (CTR NEG) according to the following plate configuration:

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1 mix + sample 1	N1 mix + sample 2	N1 mix + sample 3	N1 mix + sample 4	N1 mix + sample 5	N1 mix + sample 6	N1 mix + sample 7	N1 mix + sample 8	N1 mix + sample 9	N1 mix + CTR POS	N1 mix + CTR HSC	N1 mix + CTR NEG
B	N2 mix + sample 1	N2 mix + sample 2	N2 mix + sample 3	N2 mix + sample 4	N2 mix + sample 5	N2 mix + sample 6	N2 mix + sample 7	N2 mix + sample 8	N2 mix + sample 9	N2 mix + CTR POS	N2 mix + CTR HSC	N2 mix + CTR NEG
C	RP mix + sample 1	RP mix + sample 2	RP mix + sample 3	RP mix + sample 4	RP mix + sample 5	RP mix + sample 6	RP mix + sample 7	RP mix + sample 8	RP mix + sample 9	RP mix + CTR POS	RP mix + CTR HSC	RP mix + CTR NEG
D												
E												
F												
G												
H												

Be careful: These CTR POS and CTR HSC reagents do not contain active viral material but have to be handled with caution in a dedicated nucleic acid handling area to prevent possible qPCR contamination. Securely cap tubes after addition of each of these controls.

NB: This plate configuration can vary according to the number of tested samples. Whatever the number of these samples, positive control (CTR POS), negative control (CTR NEG) and RNA isolation control (CTR HSC) have to be included for each RT-qPCR run and primer & probe set condition.

NB: Change tips to avoid cross contamination once it is necessary.

6- Cover the plate with a suitable optical sealing foil.

7- Briefly centrifuge the plate 15-60 s at 1 000 g to remove any bubbles.

8- Meanwhile, prepare and check the run program under the following RT-qPCR conditions (compatible with most qPCR instruments):

Phase	Number of cycles	Time	Temperature	Acquisition mode
Reverse Transcription	1	15 min	50°C	-
Initial denaturation - HOT start Taq activation	1	10 min	95°C	-
Amplification	45	10 s	95°C	-
		30 s	60°C	quantification

9- Select the appropriate channel of fluorescence acquisition according to the following probe dyes information:

Primers & Probe name	Reporter Dye	Quencher Dye
N1	**FAM**	**none**
N2	**FAM**	**none**
RP	**FAM**	**none**

For further information, please contact technical support AnyGenes® via technical@anygenes.com

10- Place the qPCR plate in your qPCR instrument.

11- Start the qPCR run, following the manufacturer's recommendations and protocols.

3) Data analysis

✓ **Expected results for Quality Controls**

Quality control	Control of	Expected N1 result	Expected N2 result	Expected RP result
Positive Control (CTR POS)	qPCR efficiency & primers/probe integrity	Cq < 35	Cq < 35	Cq < 35
RNA Isolation Control (CTR HSC)	RNA isolation & potential contamination	Cq > 35	Cq > 35	Cq < 35
Negative Control (CTR POS)	qPCR mix or reagent Contamination	Cq > 35	Cq > 35	Cq > 35

If you observe different and unexpected results, please invalidate the qPCR run and re-test.

✓ **Results interpretation**
(only possible if the results with quality controls are optimal)

N1 result	N2 result	RP result	Results Interpretation
Cq < 35	Cq < 35	Cq < 35 or Cq > 35	2019-nCov amplification => positive sample
Cq > 35	Cq < 35	Cq < 35 or Cq > 35	Inconclusive Results => Repeat the qPCR and/or re-extract the sample
Cq < 35	Cq > 35	Cq < 35 or Cq > 35	Inconclusive Results => Repeat the qPCR and/or re-extract the sample
Cq > 35	Cq > 35	Cq < 35	2019-nCov not detected
Cq > 35	Cq > 35	Cq > 35	Invalid Result (possible reasons: insufficient RNA material, poor RNA quality, error in qPCR mixes)

III. Additional Informations

For further information, please contact AnyGenes® technical support via the following email address: **technical@anygenes.com**

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