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30 years' experience, packed in microtubes

2019-nCoV MONODOSE dtec-RT-qPCR Test

Genetic detection of

2019 novel Coronavirus

Edition E06 (06/2018)

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DESCRIPTION

The **2019-nCoV MONODOSE dtec-RT-qPCR Test** contains individual ready-to-use tubes containing all the components needed for **2019 novel Coronavirus** detection by using qPCR. An outbreak of pneumonia, epidemiologically linked to the Huanan Seafood Wholesale Market in Wuhan, was notified to WHO on 31th December 2019 by the Chinese Health Authorities. SARS-CoV, MERS-CoV, avian influenza, influenza and similar viruses were ruled out. Chinese scientists were able to isolate a novel coronavirus, named 2019-nCoV by the WHO and, a first genome provided on 7th January 2020, was classified as belonging to a beta-coronavirus of group 2B with at least 70% similarity in genetic sequence to SARS-CoV. Coronavirus are enveloped viruses with a positive-sense, single-stranded RNA genome belonging to the Coronaviridae family. Additional genomes of several isolates provided by 6 different labs at different places, were analysed to design a set of primers and a probe fully specific for this 2019 novel Coronavirus (2019-nCoV).

PRINCIPLE OF THE METHOD

Polymerase chain reaction (PCR) allows the amplification of a target region from a DNA template by using specific oligonucleotides. In real-time reverse transcription PCR (RT-qPCR), the RNA is first transcribed to complementary DNA by a reverse transcriptase. The accumulating amplified product can be detected at each cycle with fluorescent dyes. This increasing signal allows to achieve sensitive detection and quantification of pathogens.

KIT CONTENT

TargetSpecies MONODOSE dtec-RT-qPCR (INDIVIDUAL TUBES), contains dehydrated specific primers and probe. 60 rxn

[OPTIONAL] Internal Control, will include additionally in MONODOSE tubes: primers, probe and DNA template for an internal control of PCR.

DNase/RNase free water (GREEN CAP), 1.5 ml

LyoMix RT-qPCR (BLUE CAP), lyophilized pellet containing a DNA polymerase, retrotranscriptase, dNTPs, BSA and buffer. 1 vial, 60 rxn

LyoMix resuspension buffer (YELLOW CAP), 1 ml

Standard Template (RED CAP), dehydrated target copies.

Standard buffer (BLACK CAP), to dissolve the Standard Template. 500 µl

STORAGE CONDITIONS

Components of **2019-nCoV MONODOSE dtec-RT-qPCR Test** are stable at room temperature for transport. At arrival, if not immediately used, it should be stored at -20 ^{o}C. Individual tests are stable for one year (see expiration date on the label).

After **LyoMix RT-qPCR** (BLUE CAP) resuspension, we recommend splitting the content in several aliquots and store at -20 ^{o}C. For **Standard Template** (RED CAP) we recommend, once dissolved, store **in an exclusive box** at -20 ^{o}C.

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA isolation kit (*GPSpin* extraction/purification kits recommended)
- DNase/RNase free water (to prepare standard curve dilution)
- Micropipettes and sterile pipette tips with filters
- qPCR tubes, strips or plates
- Vortex mixer
- Spinner centrifuge
- Cooling block
- Real-time PCR device

WARNINGS AND PRECAUTIONS

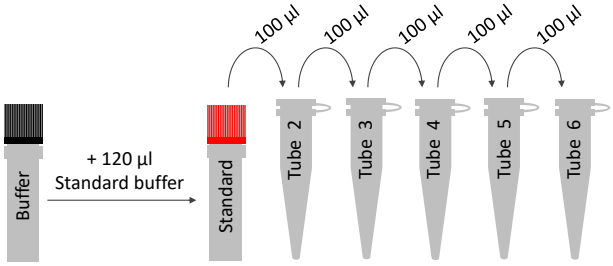
ⓘ For research use only
ⓘ To avoid possible misuse, carefully read the handbook.
ⓘ Proper training is recommended for correct operation of the kit.
ⓘ According to good laboratory practices, always wear a suitable lab coat, disposable gloves, and protective goggles.
ⓘ All the instruments used must been verified and calibrated according to the manufacturer's recommendations.

General precautions

⚠ To prevent contamination of micropipettes, use sterile tips with filters.
⚠ Micropipettes used to dilute the Standard Template should not be used for other PCR reagents.
⚠ Extract, store and prepare positive materials (samples, positive controls and PCR products) in a separately laboratory environment.
⚠ To avoid cross-contamination with the positive control, pipette it after closing reaction tubes with negative control and samples.
⚠ Work in a row and keep components refrigerated in a cooling block.
⚠ Protect the primer/probe from prolonged exposure to light.
⚠ After preparing MONODOSE tubes, run reactions immediately.

PREPARATION OF STANDARD CURVE DILUTION SERIES

1) Pipette 900 µl of **DNAse/RNAse free water** (not provided) into five tubes and label as 2 to 6
2) Pulse-spin the **Standard Template** (RED CAP), reconstitute with 120 µl of **Standard buffer** (BLACK CAP) and vortex thoroughly, label as num. 1
3) Pipette 100 µl of diluted **Standard Template** (RED CAP), into tube 2
4) Vortex thoroughly and pulse-spin
5) Change tip and pipette 100 µl from tube 2 into tube 3
6) Vortex thoroughly and pulse-spin
7) Repeat steps 5 and 6 with the tubes 4 to 6 to complete serial dilution



Standard curve dilution series	copies/µl	copies in 5 µl
Standard Template (RED CAP)	2×10^5	10^6
Tube 2	2×10^4	10^5
Tube 3	2×10^3	10^4
Tube 4	2×10^2	10^3
Tube 5	2×10	10^2
Tube 6	2	10

Pipette 5 µl of template into each well for the standard curve according to your plate set-up. The final volume in each qPCR reaction well is 20 µl.

PROTOCOL & AMPLIFICATION REGIME

Resuspend the **LyoMix RT-qPCR** (BLUE CAP) vial with 1 ml of **LyoMix buffer** (YELLOW CAP). After resuspension, 15 µl of the mastermix can be used to resuspend each MONODOSE individual tube. Vortex and add 5 µl of the sample up to a final volume of 20 µl. Vortex thoroughly and pulse-spin again.

GPS™ reagents contains BSA and are **compatible with all real-time PCR thermal cyclers, glass capillary or plate based**. Plastic of the *Generic tube* is compatible with: StepOne™, StepOnePlus™, ABI 7500 Fast, LightCycler® 96, LightCycler® Nano, CFX96™, PikoReal™ 24-well, DNA Engine® systems, MiniOpticon™ 48-12 and Opticon® 2. For other devices, please, transfer the content of the MONODOSE (20 µl) to appropriate tubes.

Take into account that the fluorescent signal must be collected by using the FAM channel for the target. If the internal control is added use the HEX channel.

	Step	Time	Temperature
	Retrotranscription	10 min	50 ºC
	Activation	1 min	95 ºC
40 Cycles	Denaturation	10 sec	95 ºC
	Hybridization / Extension and data collection[1]	1 min	**60 ºC**

1 Fluorogenic signal should be collected during this step by using the **FAM** channel for the target and by using the **HEX** channel for the internal control.

RECOMMENDED REACTION CONTROLS

These qPCR reaction controls are recommended when considering the guidelines of ISO/IEC 17025 Standard. When setting-up your qPCR protocol, select the controls considered better suits your quality system.

Negative Control (Ctrl -): Add 5 µl of DNase/RNase free water (GREEN CAP) and 15 µl of resuspended **LyoMix RT-qPCR** (BLUE CAP) to one tube. Accordingly, this reaction should be negative. A positive result may be considered as a symptom of DNA contamination in the water, making the test inconclusive. Water must be replaced.

Positive Control (Ctrl +): Prepare a standard curve dilution series as described above from the **Standard Template** (RED CAP). Add 5 µl of the standard template dilution (i.e., 2 x 10^2 copies/µl; Tube 4) and 15 µl of **LyoMix RT-qPCR** (BLUE CAP). A positive result indicates that qPCR setup is correct and works. If negative, the test should be carefully repeated after checking the thermal protocol.

Matrix Inhibition Control (M-Inh): We recommend to run reactions in parallel to test possible inhibition effects of compounds (inhibitors) present in the sample matrix. Simply, to a reaction tube, add a known amount of **Standard Template** (RED CAP) together with the sample.

Reagent	Volume
Standard template dilution (i.e., 2 x 10^2 copies/µl)[1]	5 µl
LyoMix RT-qPCR (BLUE CAP)	15 µl
Sample	5 µl
FINAL REACTION VOLUME	**25 µl**

1 Tube 4 of the curve dilutions series obtained from **Standard Template** (RED CAP)

An optimal result should show a positive signal, equal or higher (same or lower Ct) than these found for the Positive Control alone (tube 4, 10^3 copies). Inhibition may be total (negative result) or partial, observing a considerable increase in the Ct when compared to this of the Standard

Template dilution added. If inhibition is observed, a sample dilution to 1/10 may be recommended (if concentration is not close to detection limit). The matrix inhibition control is external, allowing to check the inhibition on the main target of interest.

Extraction Negative Control (ExtCtrl -): Perform an extraction according to your extraction protocol without addition of sample. Add 5 µl of extraction negative sample and 15 µl of **LyoMix RT-qPCR** (BLUE CAP). In this case, the test for DNA includes the reagents used in the extraction steps. If positive, when the **Negative Control** is negative, a contamination occurs during the extraction process. Extraction reagents must be discarded.

Extraction Positive Control (ExtCtrl +): Perform an extraction according to your extraction protocol adding the **Standard Template** (RED CAP), or DNA extracted from pure cultures into the first extraction buffer. The positive extraction control would include the effectiveness of the extraction method used. A positive result should be expected. If negative, extraction must be carefully repeated or the extraction method replaced.

INTERPRETATION OF RESULTS

The linear regression of logarithm of the copy number versus Ct gives the constants Y-intercept and slope of the standard curve (equation 1). The number of copies in the sample can be calculated based on the regression (equation 2).

$$Ct = Yinter + Slope\ x\ log(copy\ number) \quad (1)$$

$$Copy\ number = 10^{\frac{(Ct - Yinter)}{Slope}} \quad (2)$$

To obtain the sample quantification directly from the device, the Standard dilution series must be defined in the software of your qPCR device as Standard with the specified copies for each dilution (see PREPARATION OF STANDARD CURVE DILUTION SERIES, page 5). Standard curve can be defined as total copy number or copies/µl.

To refer the values obtained with qPCR to the sample material, please take into account the elution volume after extraction, the sample volume processed and any dilution performed.

Ctrl +	Sample	Ctrl -	ExtCtrl -	IC/M-Inh	Interpretation
-	+ / -	+ / -	+ / -	+ / -	Experiment fails
+	+	+	+ / -	+ / -	PCR reagents contaminated
		-	+	+ / -	Possible contamination at extraction step
			-	+	**Positive sample**
+	-	-	-	+	**Negative sample**
				-	PCR inhibition

Key symbols + and - : amplification does or not occur, respectively

VALIDATION METHODS

All batches are calibrated with a standard curve from 10^6 to 10 copies with our Standard Template. Diverse parameters are evaluated: Ct, slope, R^2 and efficiency. All this information is available in the **Quality Certification** provided to the customer by GPS™.

CONTACT INFORMATION

For any question and technical support, contact to our address support@geneticpcr.com. For quotes, orders, or new target designs, please contact orders@geneticpcr.com.

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