Product no: MBS-4100

Identifier: 2019-nCoV_E_and_RdRp Positive Control

A. Product Description

1. The product comes with 1 tube of circular plasmid DNA, which contains partial sequences of both 2019-nCoV E gene and RdRp gene. It is designed to be used as positive control in Charité/Berlin qPCR protocol (version 17th Jan 2020).
2. The product is delivered at 500µL (200,000 copies/µL) in IDTE pH 8.0 under ambient temperature.
3. For long term storage in months and years, it has to be kept at -20°C.
4. The product was designed for research use only and not for use in diagnostic procedures.

B. Preparation Before Use

1. Each microliter (µL) of product contains 200,000 copies of partial sequences from both 2019-nCoV E gene and RdRp gene within the same construct of plasmid DNA. For long term of storage, the stock solution of 200,000 copies should be kept in -20°C. Aliquot into several tubes to minimize frequency of freeze thawing.
2. The product can be diluted using 1x IDTE buffer pH 8.0 **(IDT, USA; Cat # 11050113)** according to Table 1. As positive control reaction, the diluted product of 20,000 copies is recommended for each qPCR reaction.

   Table 1: *Table of dilution to generate 200,000 and 20,000 of final copy number*

Serial dilution	Source	Initial copy number for each uL	Volume (uL)	1x TE pH 8.0 (uL)	Final volume (uL)	Final copy number for each uL
1x	MBS-4100	200, 000	1	0	1	200, 000
10x	1x	200, 000	5	45	50	20,000

3. The diluted product can be kept at 4°C and it is recommended to prepare fresh aliquots weekly.
4. Positive control reactions are mandatory for qPCR experiment. The number of qPCR reactions for each experiment depends on both (i) throughput of qPCR machine; and (ii) the number of duplicate/ triplicate reactions for each sample. Minimum duplicate reactions are required for each sample.

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	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S37	S45
B	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S37	S45
C	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	POS1
D	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	POS1
E	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43	POS2
F	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43	POS2
G	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44	NTC
H	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44	NTC

Figure 1: *The experiment above was using 96-well plate qPCR machine to analyse 48 samples, where each sample has duplicated qPCR reactions. Among 48 samples: -*

(i) 45 samples are specimen for SARS-CoV-2 detection*,*
(ii) 2 samples were two different positive control (POS1 & POS2: one for E gene, one for RdRP gene) and
(iii) 1 sample was negative control. The negative control typically also named as Non-Template Control (NTC).

5. To load 20,000 copies of plasmid positive control in each qPCR reaction, each microliter (uL) of product with 200,000 copies is sufficient for 5 duplicate reactions. As a result, each vial of product is sufficient for 2,500 duplicate reactions. If you are using 2x positive controls for each run (as illustrated in **Figure 1**), each vial will be sufficient for ~1250 runs or ~1250 experiments.

C. Quality Control

1. Sanger sequencing: the product was submitted for 1st BASE Single Pass Sequencing. The sequences were found 100% matched to the design with quality trace score of >50.

2. qPCR Assay: 200,000 copies and the diluted product of 20,000 copies was tested for each gene using both desalted and HPLC grade of primers & probes (**IDT, refer Table 2**) and THUNDERBIRD Probe One-Step qRT-PCR kit (**Toyobo, Product No. QRZ-101**) on Rotor-Gene Q (**Qiagen, Product No. 9001867**) up to 45 cycles.

   PCR Efficiency (E) for each target gene was assessed using duplicate qPCR reactions of 10-fold diluted 2019-nCoV E gene and RdRp gene, which was digested with NcoI (**Thermo Scientific, Cat# ER057**). The plasmid DNA standard of 6 magnitudes was created from 20 copies to 2,000,000 copies. Linear regression falls within the acceptable value of Slope (-3.10 to -3.59) and R^2 (> 0.95). The percentage efficiency was calculated from the slope using the formula $E = 100*(-1 + 10^{-1/slope})$. The concentration of the plasmid DNA standards was measured using Implen NanoPhotometer.

   You may click here to download the qPCR protocols for each gene.

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3. The sequences of qPCR primers and probe for each qPCR assay were provided in **Table 2**.

Table 2: Primers and Probe sequences for each qPCR Assay.

Assay	Primer/ Probe	Name	Sequence (5' to 3')	Brand	Product No.
Charité/Berlin (WHO): **E Sarbeco** Gene, ~113bp	E_Sarbeco Forward Primer	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	IDT	10006888
	E_Sarbeco Reverse Primer	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	IDT	10006890
	E_Sarbeco Probe	E_Sarbeco_P1	HEX-ACACTAGCC/ZEN/ATCCTTACTGCGCTTCG-IBFQ	IDT	Custom Probe
Charité/Berlin (WHO): **RdRP** Gene, ~101bp	RdRP Forward Primer	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	IDT	10006860
	RdRP Reverse Primer	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	IDT	10006881
	RdRP Probe	RdRP_SARSr-P2	FAM-CAGGTGGAA/ZEN/CCTCATCAGGAGATGC-IBFQ	IDT	10006886

4. The experimental data was illustrated in **Figure 2** and **Figure 3** below. For each qPCR assay, the detected Ct value shall fall within Ct20.00 to Ct35.00 to pass the Quality Control (QC).

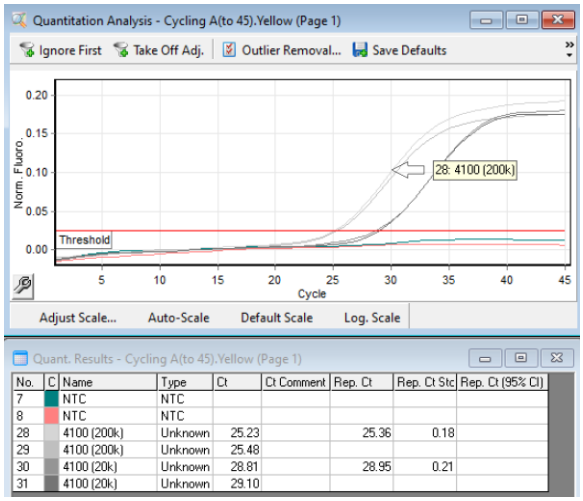

Figure 2: E Sarbeco Gene
(i) **200,000 copies:** Ct value = **25.36** and
(ii) **20,000 copies:** Ct value = **28.95**

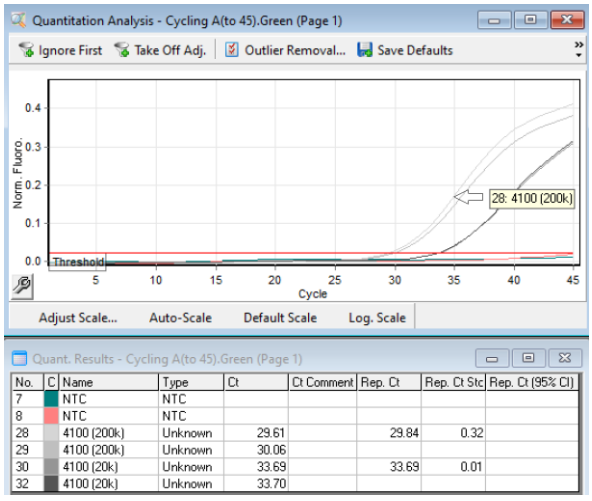

Figure 3: RdRP Gene
(i) **200,000 copies:** Ct value = **29.84** and
(ii) **20,000 copies:** Ct value = **33.69**

D. FAQ

1. How the product is being produced?

✓ The product consists of circular plasmid DNA that contains partial sequences of 2019-nCoV E Sarbeco and RdRP genes. The inserted fragments into this plasmid DNA was designed to have the binding site for primers and probes listed in Table 2, which is according to Charité/Berlin qPCR Protocol (version 17th Jan 2020): - https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf.

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- ✓ The designed DNA fragment was chemically synthesized, cloned into a holding vector, sequence verified by Sanger sequencing. Before the product is released, the purified plasmid DNA was quantified and tested with qPCR assays to target each gene.

2. Can the product use directly to generate standard curves to determine primer efficiency of any new qPCR assay adapting Charité/Berlin protocol?

- ✓ Any plasmid positive control in the format of circular dsDNA cannot be used directly to generate standard curves to determine primer efficiency in qPCR assay.
- ✓ However, the plasmid DNA can be linearized using restriction enzyme, NcoI (Thermo Scientific, Cat # ER057). After restriction enzyme digestion, the DNA has to be purified and re-dissolved using 1x TE buffer (pH8.0). The linearized DNA will have a total size of 3,199 bp. After purification, the linearized dsDNA will have new concentration, where the copy number can be adjusted using any online tool, such as http://scienceprimer.com/copy-number-calculator-for-realtime-pcr. To generate standard curves, you can try to prepare the linearized plasmid DNA from the range of 2 copies to 2,000,000 copies. You may need to buy more vials of this plasmid DNA for such optimization.

3. What is the purpose to use this product in qRT-PCR assay?

- ✓ Except for the process of reverse transcriptase, the plasmid positive control can be used to determine that the qPCR reagents, primers and probes for each qPCR assay are working properly. The loading amount of plasmid DNA for each qPCR reaction will change the Ct value accordingly. Higher amount of DNA copies will give earlier Ct value and vice versa.

4. What is the limitation of this product?

- ✓ Within the process of qPCR experiment that includes RNA extraction, cDNA synthesis and gene specific amplification: the plasmid positive control will not be able to tell when problems arise during RNA extraction and cDNA synthesis.

5. Why low copies of viral sample (e.g. 1 copy to hundreds copies) are sometimes not detected?

- ✓ It depends on assay sensitivity. You may try to increase the number of replicates or increase number of cycles, but inconsistency may still be present. The best solution is to carry out assay optimization using the choice of your preferred brand of qPCR master mix but using only the linearized plasmid DNA. You may contact us at http://www.base-asia.com/find-us, the optimization fee will be charged separately.
- ✓ If you are buying commercial viral detection kits, the detection limit of the viral copy number is usually available as part of its product information.

6. Can I dilute plasmid positive control using sterilized water?

- ✓ Water cannot prevent DNase activities. 1x TE buffer is recommended to be used to dilute the plasmid positive control DNA for better stability.

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