One Step RT qPCR kit

Cat No: RN010S

Description:

One step RT kit contains a 5x reaction buffer optimized for sensitive and specific detection of RNA species from various sources and purity backgrounds. Enzymes come separately in 20x and 200x formulations to provide flexibility and adaptations to the substrate of interest, depending mainly on the presence of PCR inhibitors (when testing direct samples without RNA extraction or difficult samples like wastewater extracts). Optimizations on SARS COV2 templates in both swab and wastewater extracts using primer/probe sets included in WHO official protocols, in single and multiplex conditions, guarantee the reproducible and efficient qPCR reactions close to the qPCR theoretical limit of detection <5 copies per reaction for pure samples and between 5-50 copies per reaction for "tough samples" *.

***"Tough samples"**: RNA samples that are produced from either direct protocol, without column extraction and washing steps, or originating from tissues or cells that have high chemical or enzymatic PCR inhibitory potential (High phenolic substances found in plant tissues or high concentration of RNases, DNases and Proteases context found in blood samples or mammalian tissue samples or fungi cells).*

Reagents supplied:

One Step RT qPCR kit, 200 rxns (Cat No: RN010S)		
Cat No	**Description**	**Tubes No.**
BR030-1	**5x One Step RT-qPCR buffer, 1 ml**	**1**
PD015-0.225	**20x Hot Start Taq DNA polymerase, 0.225ml**	**1**
RN015-0.025	**200x Reverse Transcriptase, 0.025ml**	**1**
BR056-0.05	**100 mM DTT. 0.05**	**1**
BR033- 1	**Nuclease Free Water, 1 ml**	**2**

Shelf life:
1 year upon production

Version 4/ Jan-26

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Standard 20 µl reaction:

	Component	Concentration of Stock solutions	1 reaction of 20 µl	Final Concentration/Quantity (20 µl assay)
1	**5x One Step RT-qPCR buffer**	5x	4 µl	1x
2	**100 mM DTT**	100 mM	0.2 µl	1 mM
3	**Fw primer**	10 µM	0.4 µl	0.2 µM
4	**Rv primer**	10 µM	0.4 µl	0.2 µM
5	**TaqMan Probes**	10 µM	0.2-0.4 µl	0.1-0.2 µM
6	**20x Hot Start Taq DNA polymerase**	20x	1 µl	1x
7	**200x Reverse Transcriptase**	200x	0.1 µl (0.08-0.12 µl)	1x
8	**Template RNA***	0.5-10^7 copies of target sequence/µl	1-10 µl	10 fg-500 ng
9	**Nuclease Free Water**	N/A	Till 20 µl	N/A

*** Substrate quantity: up to 10 µL/reaction is recommended for pure RNA extracts. Use up to 2 µL of substrates with high PCR inhibitors concentration.**

Alternative suggestions:

1. *After optimizing conditions and you have found the final primer/probe set you will be using, you may prepare a 10x stock and use it as such.*
2. *Sybr or EVA Green may be used instead of TaqMan probe in concentration of 0.2-2x. Start with 1x.*
3. *ROX may be added to the mix. Add according to your needs and adjust the water volume.*

When preparing a master mix, add 5 % of the reactions as excess for pipetting errors.
E.g. If someone needs to set 40 reactions then he should prepare a master mix for:

40 x 1.05= 42 reactions

If the mix is set for 37 reactions, then that would make:

37x 1.05 = 38.85 reactions,

then round up to the closest round number which is 39.

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Master mix preparation:
First add water and then continue by adding the buffer, the oligos, the 20x Hot Start Taq DNA polymerase and finally the 200x Reverse Transcriptase. While adding each enzyme to the master mix, pipette up and down for 5-10 times (to dissolve the glycerol) and then add the next one. Perform one final mix by mild vortex (1000-1200 rpm) until glycerol is fully dissolved. Aliquot the appropriate volume and add water to the negative control reactions, close the caps and then proceed to the substrate addition.

One Step RT recommended conditions for single and multiplex One Step RT-qPCR reactions:

Step 1: RT reaction. This step may be set up from 40 - 62 °C. Increase temperature for highly structured RNA targets.

Step 2: RT inactivation/ Hot Start Taq DNA polymerase activation.
Initial activation of Hot Start at 94°C for 15 minutes is very important in the case of reactions with very low PCR template copies and for obtaining the maximum efficiency in qPCR setups. DO NOT USE FEWER THAN 10 MIN!!

Step 3: Cycle denaturation. It may be set 5-15 seconds.

Step 4: Annealing/Extension step. Temperature and time depend on the primers'/probes' Tm (melting temp) and amplicons' length. The primers should be designed at Tm ~ 65 °C. Set this step at 6-10 °C below the lowest oligos' tm and use 35 seconds for amplicons up to 250 bp. Annealing extension time may be lowered in 5 seconds steps (30 or 25 seconds) depending on the limit of detection and the efficiency required for the assay in question. Lowering time period is recommended only if efficiency of the assay is not affected near the limit of detection.

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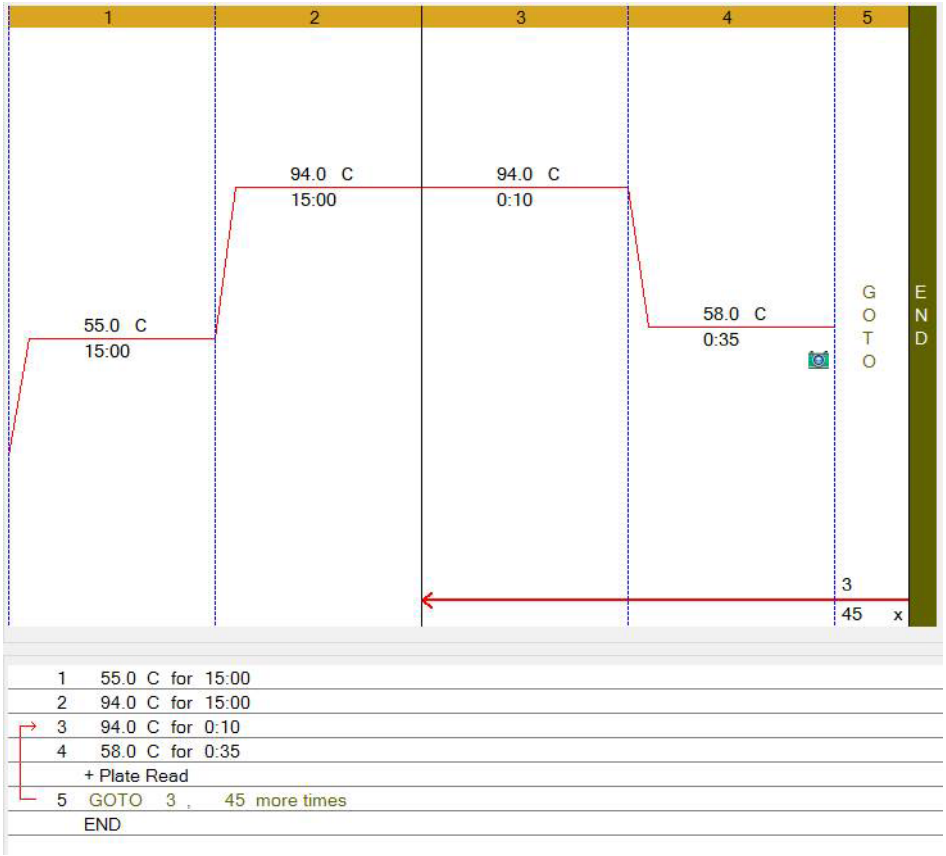

Standard protocol for single and multiplexing

Additives that improve robustness

In cases of samples with low viral content near the limit of detection (~5-10 copies/µl of wastewater sample) and/or high concentration of inhibitors one may also add extra T4gp32 protein to improve reactions' efficiency. Final concentration in qPCR reactions should be **0.05 µg/µl for T4gp32**. An example of the reaction setup including both additives may be found below:

	Component	Concentration of Stock solutions	1 reaction of 20 µl	Final Concentration/Quantity (20 µl assay)
1	**5x One Step RT-PCR buffer**	5x	4 µℓ	1x
2	**10x SARS COV2 Oligos mix**	10x	2 µℓ	1x

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3	**DTT**	100 mM	0.2 $\mu\ell$	1 mM
4	**Gp32 (Customer provided)**	10 µg/µl	0.1 $\mu\ell$	0.05 µg/µl
5	**20x Hot Start Taq DNA polymerase**	20x	1 $\mu\ell$	1x
6	**200x Reverse Transcriptase**	200x	0.1 $\mu\ell$*	1x
7	**Template RNA****	$5\text{-}10^5$ copies of target sequence/µl	2-4 $\mu\ell$	N/A
8	**Nuclease Free Water**	N/A	Till 20 $\mu\ell$	N/A

Recommended suppliers:

- For **Gp32** use any provider that is compatible with molecular biology applications. The inhouse assays have been performed with cat number **M300S or L from New England Biolabs** (T4 Gene 32 Protein)

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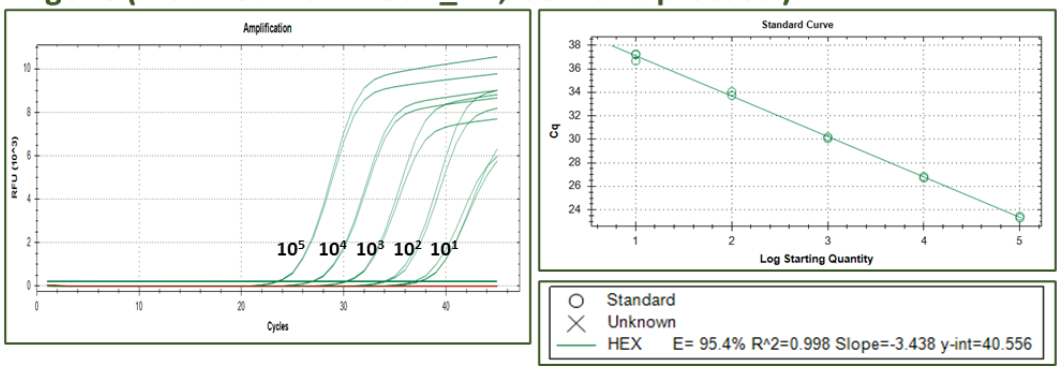
Example: SARS COV 2 standard EURM019

N gene (modified from nCOV_N2, USA CDC protocol)



E gene (modified from the Charité protocol)

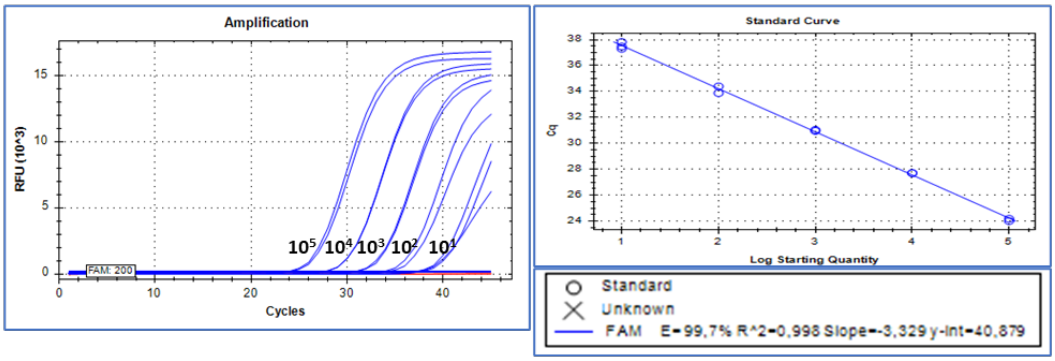


Figure 1. SARS COV2 standard curves produced by One Step RT qPCR kit (Cat No: RN010S). The standard curves were produced by analyzing the EURM019 SARS COV 2 standard over 5 logs. Standard dilutions were prepared, having as a given that the EURM019 stock solution contains 10^8 copies of SARS COV2 chimeric transcripts/µl (detailed info for EURM019 used may be found at https://crm.jrc.ec.europa.eu/p/EURM-019). Since the quantification of the EURM019 standard, performed by digital droplet PCR by the manufacturer, produced results between 6 - 7.6 x 10^7 copies/µl depending on the primer set used, the actual number of copies that were introduced in each reaction may be 24-40% less than indicated in the figure.

Reactions were set in duplicates for 10^5-10^2 copies and in triplicates for 10 copies.
The Baseline was manually set to 200 Relative Fluorescent Units.
The results were automatically produced by CFX Maestro software. The reactions were incubated to either CFX96 or OPUS96 qPCR machines with very similar results.

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Details regarding the primer sets used may be found at https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf

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