EQ Flu A/B One Step RT qPCR kit for wastewater, 200 rxns

Cat No: RN017S

Description:

EQ Flu A/B One Step RT qPCR kit for wastewater contains a 5x reaction buffer optimized for sensitive and specific detection of Influenza A virus and Influenza B virus RNA species from various sources and purity backgrounds. Enzymes come separately in 20x and 200x formulations to provide flexibility and allow user to optimize depending on the purity of the substrate of interest (mainly on the presence of PCR inhibitors). The 10x Flu A/B Oligos mix contains a combination of primers and probes optimized for the detection of Influenza A virus and Influenza B virus at either FAM or HEX channel respectively. Optimizations on wastewater extracts samples guarantee the reproducible and efficient qPCR reactions close to the expected limit of detection (<10 copies per reaction).

Table 1.Reagents supplied:

EQ Flu A/B One Step RT qPCR kit for wastewater, 200 rxns (Cat No: RN017S)		
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**
BR067-0.425	**10x Flu A/B Oligos mix, 0.425 ml**	**1**
BR033-1	**Nuclease Free Water, 1 ml**	**2**
BR068-0.024	**qPCR Enhancer, 0.024 ml**	**1**
BR056-0.05	**100mM DTT, 0.05 ml**	**1**
BR070-0.05	**FluA/B positive control RNA IVT (**quantity to be defined): it is set so it contains similar quantities for FluA and FluB RNA IVT (provided by Prof. Dovas laboratory, AUTH). Cq values **obtained is ~27.5** for both FAM and HEX with the base line set 180 RFUs in CFX 96 qPCR machine.	**1**

Shelf life:
2 years post production.

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Table 2. 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-PCR buffer**	5x	4 µl	1x
2	**10x FluA/B Oligos mix**	10x	2 µl	1x
3	**100 mM DTT**	50 X	0.2 µl	1X
4	**qPCR enhancer**	200 X	0.1 µl	1 X
5	**20x Hot Start Taq DNA polymerase**	20x	1 µl	1x
6	**200x Reverse Transcriptase**	200x	0.1 µl*	1x
7	**Template RNA****	$5\text{-}10^5$ copies of target sequence/µl	2-4 µl	N/A
8	**Nuclease Free Water**	N/A	Till 20 µl	N/A

***0.08-0.12 µl may be added depending on the substrate used. For Reference Labs wastewater extracts analysis, 0.1 µl has been used extensively used. Do not add more than needed, because PCR inhibition effect may be caused by the excess of Reverse Transcriptase enzyme in the reaction.**

****3 µl of RNA extracts is recommended. In case of high concentration of PCR inhibitors, perform 1:10 dilution of the original wastewater extract sample and add 2-4 µL per reaction. 2 µL of wastewater extracts from Reference Labs have been extensively used in our analysis**.

Optional: Depending on the wastewater quality, the region or the season collected, treatment of wastewater extracts with PCR inhibitors removal kits like the ones provided by ZYMO Research (https://zymoresearch.eu/collections/onestep-pcr-inhibitor-removal-kits), may be important to obtain accurate, quantifiable results. In order to verify the necessity of this type of treatment, test 4 independently extracted samples, collected over 2-4 weeks, and compare the quantification of the 1:10 dilution of either treated or untreated sample. If the difference obtained is lower than 30 % then that treatment may be omitted from the analysis.

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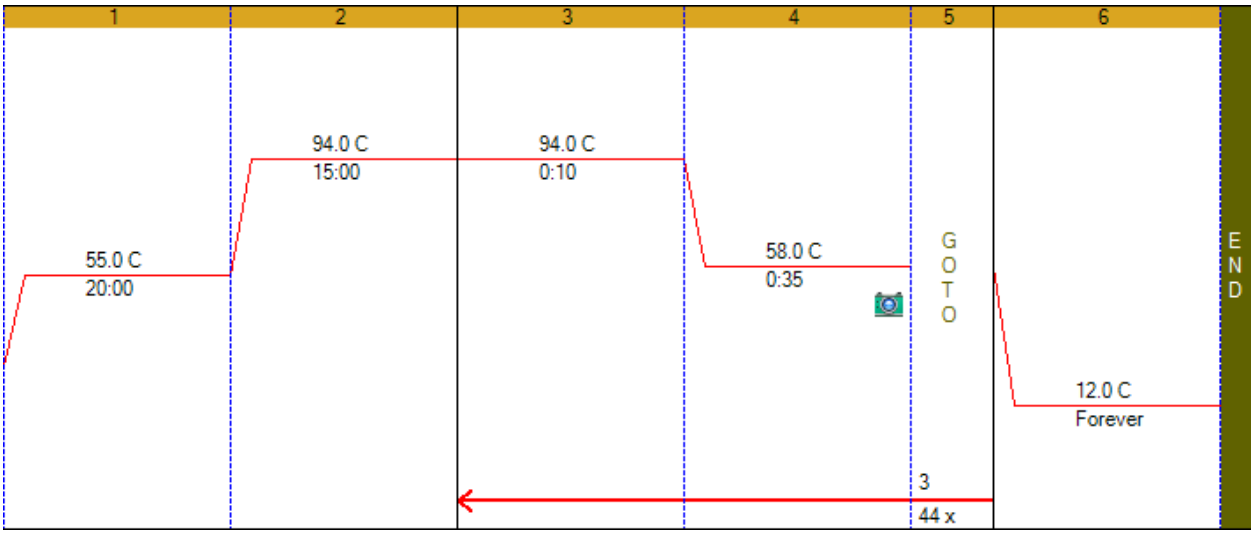
One Step-RT recommended conditions.



Step 1: RT reaction. Important step to ensure success in detecting and quantifying efficiently low level transcripts. RT step may be increased further to 25 minutes to further enhance specificity. But it is not necessary in all cases. It is not recommended to compare levels between runs that have been set with different RT times. Test in the beginning and proceed with the same program each time to ensure quality comparisons.

Step 2: RT inactivation/ Hot Start Taq DNA polymerase activation.

Initial activation for 15 minutes at 94°C is important for reactions with low PCR template copies and for obtaining maximum efficiency in qPCR setups. DO NOT USE FEWER THAN 10 min!!

Step 3: Cycle denaturation.

Step 4: Annealing/Extension step. The recording is on FAM and HEX channel.

After the analysis finishes, set the baseline in the area where maximum efficiency is obtained. For Bio-Rad platforms, set at 150-350 Relative fluorescent units (180 RFUs is our standard practice). For other platforms adjust according to your standard practice.

(Optional/ When applicable) Quantify samples with or without PCR inhibitor removal treatment and see whether there is a difference greater than 30 %. If there is, then the PCR inhibitor removal kit may be necessary before the RT-qPCR analysis for the accurate quantification of the viral load in the wastewater sample. If in both cases the result is similar, then the PCR inhibitor removal step may be omitted.

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The Routine/minimal assay

After the initial run, the routine assay for wastewater analysis may contain:

i. **A standard curve of 5 logs in duplicates of 10^5-10 copies/reaction** → duplicates for 10^5-10^3 triplicates for 10^2 and 10 copies reactions**. 12 reactions**
ii. **3 µl of undiluted waste -water sample** → At least quadruplicates especially when level of FluA or FluB are less than 10 copies per µl. 6 replicates may be used instead for very low RNA levels. Ideally, **4 reactions.**
iii. **2 negative control reactions→ 2 reactions**

18 reactions

Master mix preparation

First add water and then continue by adding the buffer, the oligos, the DTT, the qPCR enhancer and then the 20x Hot Start Taq DNA polymerase. Mix briefly by pipetting and mild vortex (~ 1000 rpm) for 5 seconds to dissolve glycerol and finally add the 200x Reverse Transcriptase. Repeat mixing by finger tapping and mild vortex and spin to collect and disperse bubbles (spin at 2000 g for 5-10 sec). While adding each enzyme to the master mix, pipette up and down for 5-10 times (to dissolve glycerol) and then add the next one. Perform one final mix with a mild vortex (1000-1200 rpm) until glycerol is completely dissolved. Aliquot the appropriate volume and first , add water to the negative control reactions and close the caps. Then proceed to the samples addition and close the caps and finally add template to the standard curve reactions and close the caps.

Always calculate an additional 5% of each component when calculating volumes for master mix preparation to avoid pipetting errors. E.g. if you wish to set 20 reactions, then you should multiply the volume needed for one reaction by 21 for each component. If you wish to prepare 10 reactions, then multiply by 10.5 etc.

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 qPCR enhancer in steps of 0.01 µL. Up to 0.12 µl / reactions have been shown to help in some cases. The kit provided quantity (24 µl) suffices for at least 200 reactions with 0.12 added given that no losses due to pipetting occur. We strongly suggest testing the recommended quantity mentioned in Table 2 before increasing the amount per reaction.

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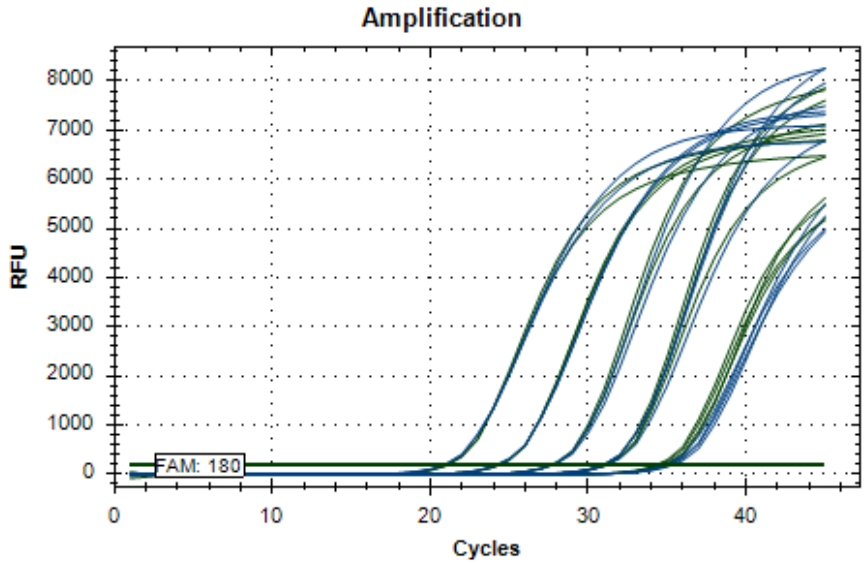
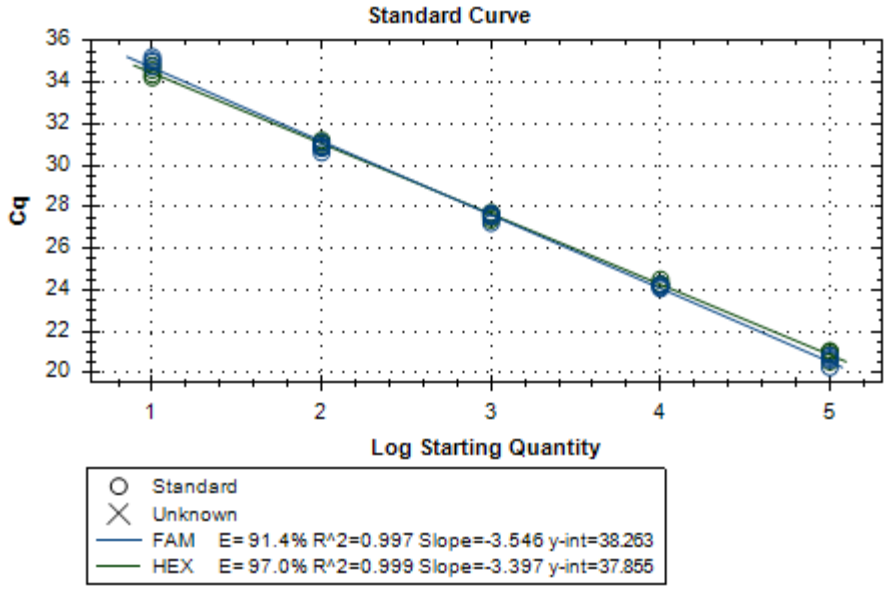
Appendix 1





10^5 to 10 copies of FluA and FluB IVT RNA based on the assumption that the stock provided contains 10^8 copies/µl of either FluA or FluB /µl. 3 µl were used as template. The result has been produced with kit RN017S, lot: RN017 0100.

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