Quantification by Droplet Digital PCR (ddPCR) V.1

Adélaïde Roguet[1]
[1]UWM

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mclellan lab
M McLellan Lab

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PARENT PROTOCOLS

In steps of
Concentrate viruses from sewage using HA filters
Prepare bovine coronavirus (BCoV) solution
Concentration of viruses from sewage using HA filters
Prepare bovine coronavirus (BCoV) solution

GUIDELINES

This protocol is for quantification for SARS-CoV-2 RNA in wastewater using one-step RT-ddPCR.

BEFORE STARTING

Cleaning working space by wiping down using 70% ethanol and RNase away.
Prepare an ice bucket. Take out reagents from -20ºC freezer, , including master mix, reverse transcriptase, DTT, primers, probes and other reagents may need. Thaw reagents on ice for half an hour.
Take out RNA samples from -80ºC and thaw on ice before setting up reaction.

1 When all reagents are thawed on ice, vortex Supermix, Reverse transcriptase and DTT throughly for 30 seconds. Vortex to mix primers and probes stocks.

2 Prepare the reaction matrix (for one well, beside sample RNA) according to the table below. Prepare Use a low-binding tube of appropriate volume to mix all the components according to the reaction numbers. Always include extra wells when setting up reaction to avoid potential volume shortage caused by pipetting.

Component	Volume per reaction, uL	Final concentration
Supermix	5.5	1x
Reverse transcriptase	2.2	20 U/uL
300 mM DTT	1.1	15 mM
Primer mix (forwad + reverse)	1.1	900 nM
Probe	1.1	250 nM
RNase-free water*	5.5	/
Total	16.5	/

* Note: Water volume can be replaced accordingly by another assay (e.g., duplex assay), or another RNA template (e.g., inhibition test).

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