

iLAMP Nov-CoV19 Detection Kit Protocol report 2st April 2020

ThermoFisher Quantum Studio 5 (384 well)

Methodology:

MIX (hold 5 minutes at RT.):

- 8 ul real time lamp pre mix
- 2 ul CTR 8stock concentration);

Thermal cycler setting (Quantstudio5):

- chemistry: SYBR GREEN
- Passive reference: NONE
- 384 well block

Set the cycler:

- 50°C 5"
- 65°C 0:30" (60 cycle)

Amplification of CTR1 is 24,67 CT.

(CFX96TH, BioRad, 96 well).

Experiment n.1 performed on 2st-04-2020

Reagents:**CTR+ 2nd tube** (5 ul stock concentration, dilution 1:5 of stock concentration)**CTR+ 1st tube** (5 ul stock concentration, dilution 1:5 of stock concentration)**Methodology:**

MIX:

- 20 ul real time lamp pre mix
- 5 ul CTR (stock concentration); 1ul (dilution 1:5 of stock concentration) + 4ul water nuclease free

Thermal cycler setting (CFX96TH, BioRad):

- chemistry: SYBR GREEN
- Passive reference: NONE
- 96 well block

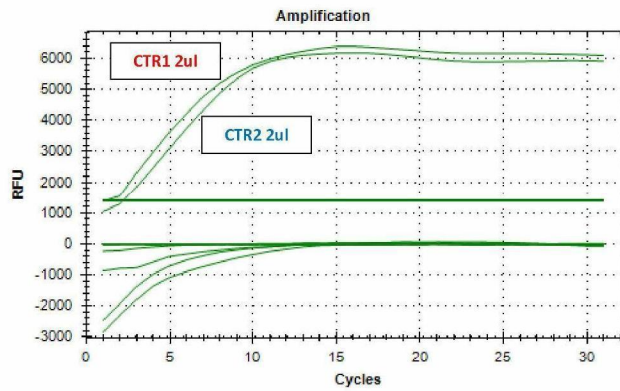
Set the cycler:

- 50°C 5"
- 70°C 0:30" (30 cycle)

*Amplification of CTR1 2ul is 2,23 CT.**Amplification of CTR1 1ul is 1,19 CT.**No Amplification of both of CTR+ 2nd tube (dilution 1:5 of stock concentration) and CTR+ 1st tube (dilution 1:5 of stock concentration).**NB: CTR+ 2nd tube has been previously shown with an amplification plot (see experiments of 31th march 2020).**NB: CTR+ 1st tube has been previously shown with an amplification plot (see experiments of 1th april 2020).*

Sample Name	CT
CTR1 2ul	2,23
CTR2 2ul	1,19
CTR1 1ul (dilution 1:5 of stock concentration)	Undetermined
CTR2 1ul (dilution 1:5 of stock concentration)	Undetermined

Negative control	Undetermined
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**Conclusions:**

- 1) With Thermofisher changing conditions and increase the volume mix, the CT values are under the detection of 25 CT. The kit is stable and is performing well.
- 2) With the BioRad CF96TH instrumentation, the sensitivity of the KIT seems to be higher than the analyzes carried out with Quantstudio5 machine with an amplification (around two CT). We are now in the process to run the experiments on the Biorad CFX96.

(10)(2a) 2st April, 2020