Dec 15, 2020

CuPCR SARS-Cov-2

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1 Works for me dx.doi.org/10.17504/protocols.io.bpqwmmxe

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ABSTRACT

RNA based diagnostic test of different human samples (nasopharyngeal swabs, saliva) to detect SARS-Cov-2. The test requires RNA/DNA extraction and a follow-up RT-PCR (reverse transcriptase polymerase chain reaction). The test covers the N-gene and RdRp-gene and covers a human control as well. The human control safeguards the validity of sample taking and test procedure.

EXTERNAL LINK

http://www.cubedx.com

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PROTOCOL CITATION

B Ronacher, Christoph Reschreiter 2020. CuPCR SARS-Cov-2 . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpqwmmxe

EXTERNAL LINK

http://www.cubedx.com

KEYWORDS

RT-PCR SARS-Cov-2, N-gene, RdRp-gene, covid19, corona test

CREATED

Nov 15, 2020

LAST MODIFIED

Dec 15, 2020

PROTOCOL INTEGER ID

44534

PARENT PROTOCOLS

In steps of
Viral Testing Inc In Collaboration with Cube Dx GmbH

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ABSTRACT

RNA based diagnostic test of different human samples (nasopharyngeal swabs, saliva) to detect SARS-Cov-2. The test requires RNA/DNA extraction and a follow-up RT-PCR (reverse transcriptase polymerase chain reaction). The test covers the N-gene and RdRp-gene and covers a human control as well. The human control safeguards the validity of sample taking and test procedure.

RT-PCR test 30m

1 **00:30:00** RNA / DNA extraction 30m

Kingfisher Flex 96
RNA / DNA extraktor
Thermo Fisher Scientific **5400630**
RNA/DNA extraktor processing up to 96 samples in a single run.

100µl eluation buffer (e.g. water) containing RNA / DNA from the sample

2 **01:20:00** RT-PCR 1h 20m

Prepare mastermix:
5µl of primer mix + 5µl of enzyme mix (per sample)

Put mastermix and sample into PCR tube:
10µl of mastermix + 10µl of sample (eluate)

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Select (or program) RT-PCR protocol:
- ☐ 2 minutes 50 °C
- ☐ 2 minutes 95 °C
- ☐ 45 cycles:
  - 5 seconds 95 °C
  - 1 minute 60 °C
  - Scan
- ☐ 25°C for hold

Load samples and start PCR

Analyse amplification curves:
- Human control: ct < 34 --> valid
- N-gene SARS-Cov-2: ct > 0 --> positive
- RdRp-gene SARS-Cov-2: ct > 0 --> positive

qTower3
Realtime PCR
Analytic Jena **844-00563-2**

Amplification curves for: human control, N-gene of SARS-Cov-2, RdRp-gene of SARS-Cov-2

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