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qPercerus
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ABSTRACT

Protocol for qPercerus SARS-CoV-2 Test (attached document is giving more details).

Final product will be a commercial kit that is consisting of four components (lysis buffer, primer/probe mix identification, primer/probe mix confirmation, qPCR master mix). The initial test for SARS-CoV-2 is the identification assay (FAM assay for S gene with HEX assay as internal control) for patient screening. The confirmation assay (FAM assay for Orf3a with HEX assay as internal control) is used to re-evaluate the results.

ATTACHMENTS

[qPercerus SARS-CoV-2.pdf](#)

MATERIALS

qPercerus Lysis/Extraction Buffer
qPercerus Primer-Probes Identification Assay
qPercerus Primer-Probes Confirmation Assay
qPercerus qPCR Mastermix

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Created: Nov 26, 2020

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PROTOCOL integer ID:
44922

Keywords: SARS-CoV-2
Detection, RT-qPCR

1 Add 200 µL Lysis Buffer to reaction tube

- 2 Add **Nasopharyngeal** or **Throat Swab** to **Lysis Buffer** and **swivel** for 00:00:10 to lysate and inactivate virus. Remove and **discard** the **Swab** afterwards. 10s

Note

If **Nasopharyngeal** and **Throat Swab** are available from the same patient, the sensitivity can be increased by using both in one qPercerus reaction.

- 3 Briefly vortex and incubate at Room temperature for 00:10:00 10m

Note

Sample is stable in **Lysis Buffer** for up to 48:00:00 at Room temperature allowing for storage and transport.

- 4 Mix RT-qPCR Master Mix for first **Identification Assay** with 10% surplus and distribute on suitable qPCR microtiter plate:

10 µL ID Primer/Probe Mix per reaction

5 µL Master Mix per reaction

- 5 Add virus lysate to each well on qPCR microtiter plate:

10 µL Virus Lysate

- 6 Briefly spin down qPCR microtiter plate

1000 x g, Room temperature, 00:00:30

- 7 Run **One-Step RT-qPCR** with the following temperature protocol:

50 °C 00:10:00 reverse transcription

95 °C 00:03:00 RT Inactivation/ Polymerase activation

For **45 cycles**:

95 °C 00:00:10

60 °C 00:00:30 acquiring on FAM and HEX

FAM channel detects **SARS-CoV-2** related fluorescence

HEX channel detects **human Internal Control** related fluorescence

8 Evaluate **Cqs** of **HEX** channel for swab validity:

Cq of **HEX** <30 sample valid for analysis

Cq of **HEX** >30 inconclusive -> perform **Confirmation Assay** for validation

Evaluate **Cqs** of **FAM** channel for SARS-CoV-2 presence:

Cq of FAM <35 positive for SARS-CoV-2

40 > Cq of FAM >35 inconclusive -> perform **Confirmation Assay** for validation

Cq of FAM >40 or NA negative for SARS-CoV-2

Note

If national testing regulations demand validation of positive SARS-CoV-2 presence on an independent target sequence, perform **Confirmation Assay** for Cq of FAM <35 .

9 Mix RT-qPCR Master Mix for first **Confirmation Assay** with 10% surplus and distribute on suitable qPCR microtiter plate:

10 µL CONF Primer/Probe Mix per reaction

5 µL Master Mix per reaction

10 Add virus lysate to each well on qPCR microtiter plate:

10 µL Virus Lysate

11 Briefly spin down qPCR microtiter plate

1000 x g, Room temperature, 00:00:30

12 Run **One-Step RT-qPCR** with the following temperature protocol:

50 °C 00:10:00 reverse transcription

95 °C 00:03:00 RT Inactivation/ Polymerase activation

For **45 cycles**:

95 °C	00:00:10
60 °C	00:00:30 acquiring on FAM and HEX

FAM channel detects **SARS-CoV-2** related fluorescence

Cy5 channel detects **human Internal Control** related fluorescence

- 13** Evaluate **Cqs** of **HEX** channel for swab validity:
- Cq of **HEX** <30 sample valid for analysis
  - Cq of **HEX** >30 sample invalid, repeat sampling of

Evaluate **Cqs** of **HEX** channel for SARS-CoV-2 presence:

Cq of **FAM** <40 positive for SARS-CoV-2

Cq of **FAM** >40 or NA negative for SARS-CoV-2