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qPercerus

Forked from qPercerus

Robert P Loewe[1], Benedikt Kirchner[1]

[1]GeneSurge GmbH

1 Works for me dx.doi.org/10.17504/protocols.io.bp42mqye

Benedikt Kirchner

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

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PROTOCOL CITATION

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FORK NOTE

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ATTACHMENTS

qPercerus SARS-CoV-2.pdf

MATERIALS TEXT

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

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.

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

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

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

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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 aquiring 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

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 aquiring on FAM and HEX**

FAM channel detects **SARS-CoV-2** related fluorescence
Cy5 channel detects **human Internal Control** related fluorescence

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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

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