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Cellex qCoV Protocol V.1

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1 Works for me dx.doi.org/10.17504/protocols.io.bku5kwy6

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ABSTRACT

Cellex's qCOV assay is a viral enzyme activity assay, which detects the activity of coronaviral protease 3CL (chymotrypsin-like protease) in 15 minutes with sensitivity equivalent to 36 cycles of RT-PCR using reagents that are inexpensive and simple to mass produce. We engineered a thermostable firefly luciferase with a 3CL cleavage sequence (mutant luciferase or mLuc). Presence of 3CL enzyme in the sample leads to inactivation of luciferase activity as indicated by reduced light signal. Since luciferase/luciferin is one of the most efficient light generation systems – as few as 2000 molecules could be detected – and 3CL is extremely active with high reaction velocity, this detection system is highly sensitive for detection of coronavirus.

Until now detection of acute SARS CoV-2 infection has required either molecular assays or antigen assays to detect viral nucleic acids or antigens. These assays have not provided an adequate diagnosis solution to COVID-19 pandemic management. The qCOV assay isa completely different detection method and assay: the detection of viral enzyme activity as a means for simple (without sample preparation), rapid (15 min), and sensitive (equivalent to 36 cycles of RT-PCR) detection of active coronavirus infection.

The assay is a simple homogeneous assay without sample preparation, involving 1) addition of 25 uL Reagent I to 100 uL sample (e.g., throat swab extract) and incubation for 10-15 minutes to allow 3CL digestion, and 2) addition of 25uL Reagent II to enable luciferase reaction and production of light signal. It can be run at point of care settings or in a laboratory in a high throughput fashion. Thousands of samples can be tested in a day with a simple luminometer or other instruments that have luminscence capabilities which are already widely available in nearly every clinical laboratory.

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Enzyme assay, 3CL protease, homogeneous assay, rapid

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MATERIALS TEXT

A. Instruments

a. Luminometer or an analyzer capable detecting light signal (glow or flash light)

Required specifications:
1) Wavelength: capable of detecting light signal with wavelength of 550 -570 nm
2) Sensitivity: picomoles of firefly luciferase or picomolesof ATP
3) Linearity: 3 logs or better

b. Oven:The assay temperature is 30 ± 5oC. An oven capable of maintaining temperature between 25oC and 35oC is needed if the analyzer does not have temperature control capability and the ambient temperature is below 25oC.

B. Materials

a. qCOV Enzyme Rapid Test kit, which consists of following components:

Kit Component	Description
Sample Buffer	For extraction of throat swabs
Reagent I	Contains mLuc digestible with 3CL protease
Reagent II	Reagents enabling bioluminescence reaction
Positive Control	Sample buffer solution containing 3CL protease

b. Black or other microwell plates suitable for luminescence assays

c. Sample collection and extraction supplies

Sample Collection

1 **Prep for Sample Collection:** Prefill 0.25 mL of sample buffer to a sample extraction vial. Place the vial in a test tube rack.

2 **Sample Collection:** Use a throat swab to collect a throat swab specimen, making sure that epithelial cells are collected from the oropharynx. Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx at least 5 times and avoid touching the tongue, teeth, and gums.

3 **Sample Extraction:** Insert the swab into the sample extraction vial. Rub the swab against the tube wall. While squeezing the tube wall against the swab, slowly remove the swab from the vial. The extract should be frozen if not used immediately for testing.

Reaction

4 **Check the ambient temperature.** If it is below **25 °C** , turn on the oven and set the temperature to **30 °C** .

5 **Bring all reagents to room temperature.**

6 **Set up a run:**

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6.1 Use the sample buffer as the negative control. If the sample is prepared in a sample buffer different from that of the qCOV Enzyme Rapid Test, the sample buffer should be used as the negative control. For example, if the sample in VTM is used, VTM solution should be used as the negative control.

6.2 Run two negative controls and one positive control for each batch run.

6.3 One of negative controls should be placed at the beginning of the run while the other should be placed at the end of the run.

7 **Add 100 µl of sample or control to a well.**

8 **Add 25 µl of Reagent I to each well** and transfer the plate to the oven; incubate for **00:15:00** .

Signal Measurement

9 **Add 25 µl of Reagent II to each well;** insert the plate to the luminometer and complete signal measurement within **00:10:00** .

Test Result Interpretation

10 **Calculate the relative activity (RA) of inhibition as follows:**

RA = (NC 1 + NC2) ÷ 2 ÷ Sample

The signal is in relative light units (RLU)

11 **Use interpretation guide below to interpret result:**

RA Value	Interpretation	Recommended Action
≥ 2.00	Positive (Active infection)	Confirmation by RT-PCR
1.50	Negative (No infection)	No action

*The indeterminate results may be interpreted as positive or negative depending on the sample types and / or prevalence of SARS CoV-2 in the community.

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