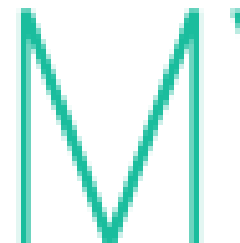


Aug 31, 2020

Mmolecular COVID-19 Extraction-Free Direct-One-Step Fast Cycling Protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.bkjpkmn>



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Protocol status: In development

We are still developing and optimizing this protocol.

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Last Modified: August 31, 2020

Protocol Integer ID: 41295

Keywords: prior rna purification step, time analysis of target rna, target rna, rna, qpcr, swabs without the requirement, step rt, step fast cycling protocol

Disclaimer

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Abstract

The Direct One-Step RT-qPCR COVID-19 Test Kit is designed for quantitative real-time analysis of target RNA directly from, swabs without the requirement of any prior RNA purification steps.

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles stable at 4 °C for up to 4 weeks

Shelf Life: 12 months

Form: liquid / lyophilized



Well plate preparation

5m

- 1 Pre-mix the reagents for 81 wells 81 μL total volume

2m

40.5 μL Direct reaction mix

3.25 μL Enzyme mix

8.25 μL Primers and probes mix

29 μL PCR grade water

- 2 Pipette the mix onto the 81 well plate.
To gain speed, use a pipette-ing robot.

3m

Sample processing

9m 30s

- 3 Place the swab brush into a 1.5ml microcentrifuge tube containing

135 μL PCR-grade water and 15 μL PBS (10x conc) .

Rotate the brush 5-10 times.

Squeeze the brush and remove it from the tube.

- 4 Centrifuge at 12000 x g for 3 min at room temperature

3m

- 5 Discard the supernatant.

Add 4.5 μL PCR-grade water and 0.5 μL Extraction Buffer

Briefly flick the sample.

1m

- 6 Incubate for 3 min at room temperature for lysis and release of RNA.

3m

- 7 Centrifuge briefly and transfer 0.25 μL sample to the well plate.

Pipette up/down to make sure the sample has fully released from the pipette.

1m

- 8 Cover the sample with 1 μL mineral oil

30s

Cycling

15m 30s



- 9 Load the well plate into the cyclor and move the chamber door down.
Click run.

15m 30s

Run with parameters:

- RT 50C for 3 min
- Denaturation 95C for 0s (maximum speed)
- Annealing 60C for 0s (maximum speed)