Aug 31, 2020

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

steve.puts [1]

[1]Mmolecular.com

In Development dx.doi.org/10.17504/protocols.io.bkjpkumn

Coronavirus Method Development Community | Mmolecular

steve.puts

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

DOI

dx.doi.org/10.17504/protocols.io.bkjpkumn

PROTOCOL CITATION

steve.puts 2020. Mmolecular COVID-19 Extraction-Free Direct-One-Step Fast Cycling Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkjpkumn

CREATED

Aug 31, 2020

LAST MODIFIED

Aug 31, 2020

PROTOCOL INTEGER ID

41295

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the

information contained in or linked to this protocol or any of our Sites/Apps and Services.

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. 3m
To gain speed, use a pipette-ing robot.

Sample processing 9m 30s

3 1m

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. 1m
Add **4.5 µl PCR-grade water** and **0.5 µl Extraction Buffer**
Briefly flick the sample.

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. 1m
Pipette up/down to make sure the sample has fully released from the pipette.

8 Cover the sample with **1 µl mineral oil** 30s

Cycling 15m 30s

9 Load the well plate into the cycler and move the chamber door down. 15m 30s
Click run.

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

Citation: steve.puts (08/31/2020). Mmolecular COVID-19 Extraction-Free Direct-One-Step Fast Cycling Protocol. https://dx.doi.org/10.17504/protocols.io.bkjpkumn