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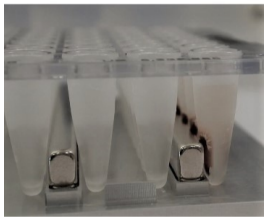
Manual Silane magnetic bead-based high throughput protocol for SARS-CoV-2 RNA extraction V.1



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Coronavirus Method De...

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We use this protocol and it's working

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Abstract

The COVID-19 pandemic has presented an unprecedent challenge to develop and validate testing tools for urgent disease diagnosis. Current testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) via molecular diagnostics is woefully inadequate due to myriad issues with cross-entity coordination, reagent availability, and labor- and time-intensive protocols. The bottleneck in the workflow from patient sampling to diagnosis is not in the identification of viral genetic material (RT-qPCR) but in the collection, processing, and purification of the viral genetic material (patient sampling and RNA extraction). The majority of tests performed in laboratories lacking sophisticated automation require individual sample processing that severely limits the possibilities of scale-up. Thus, throughput depends on the availability of trained operator person-hours.

We have developed a protocol for SARS-CoV-2 RNA extraction based on silane ferrimagnetic beads that is easily scaled up to multiwell culture plates with the processing done using multichannel pipettes. This protocol significantly reduces the hands-on time per sample and increases throughput such that an individual operator can process 100-200 samples per hour.

Materials

MATERIALS

- Buffer RLT Plus **Qiagen** **Catalog #**1053393
- 80% Ethanol
- DynaMag™-96 side skirted magnet **Life Technologies** **Catalog #**12027
- 100% Ethanol
- DynaMag™-2 Magnet **Thermo Fisher** **Catalog #**12321D
- Graduated Safelock Microcentrifuge Tubes, 2.0mL **Thermo Fisher** **Catalog #**3458
- Dynabeads™ MyOne™ Silane **Thermo Fisher** **Catalog #**37002D
- 10 mM Tris pH7.5
- Swabs
- 96-Well Semi-Skirted Plates **Thermofisher** **Catalog #**AB0900
- Polystyrene Solution Reservoirs **Labdex** **Catalog #**03-103PSRL
- Pippette Tips GP LTS 1000 ul (filter) **Mettler Toledo** **Catalog #**30389272
- Pippette Tips GP LTS 200 ul (filter) **Mettler Toledo** **Catalog #**30389276
- Pippette Tips GP LTS 20 ul (filter) **Mettler Toledo** **Catalog #**30389274
- Pippette Tips GP LTS 20 ul **Mettler Toledo** **Catalog #**30389270
- LTS Pipette L-1000XLS **Mettler Toledo** **Catalog #**17014382
- Multi Pipette L8-200XLS **Mettler Toledo** **Catalog #**17013805
- Multi Pipette L8-20XLS **Mettler Toledo** **Catalog #**17013803
- Vacuum station
- Biosafety Level 2 Laminar Flow Hood

Safety warnings

Biosafety

- Be sure to wear the appropriate equipment: lab coat, two pairs of gloves, face mask and glasses.
- Use UV light to inactivate any remaining viral particles inside the laminar flow hood.

Before start

Reagent preparation and considerations

SAMPLE COLLECTION

- Aliquot 800 µl of RLT plus buffer into 2 mL safe-lock ED tubes
- Use safe-lock tubes for sample collection and transport to prevent sample leakage

RNA EXTRACTION

- Pre-heat an aliquot (~10 mL) of 10 mM Tris-Cl pH 7.5 to 37C.
- Prepare fresh 80% ethanol solution
- Use multichannel pipettes to increase the throughput (we use Rainin pipettes)
- Use filter tips for the entire process except for the aspiration steps
- If possible, install a vacuum station with an 8-channel adaptor

Sample collection

1 Following oropharyngeal and/or nasopharyngeal swab collection, place the swab directly into a 2 mL safe-lock tube containing 800 µl of RLT-plus buffer (Qiagen)

** When collected in RLT plus the viral particles become inactive. This provides further biosafety for the process. However, samples should be always considered as extremely biohazardous and handled with extreme caution inside a Level 2 biosafety hood*

2 Cut the swab's stem and close the tube.

3 If possible, store samples at 4C or -20C.

** We have seen that when placed in RLT plus buffer the RNA integrity of the samples is preserved at room temperature for 24-48 hours.*

Sample transfer into deep 96-well plates

30m

4 Let samples thaw for 20 minutes at Room Temperature (if transported or stored frozen).

** All the process should be performed inside a Biosafety Level 2 Cabinet following the WHO guidelines for processing SARS-CoV-2 samples.*

** Use Eppendorf deep-well plates with capacity for 250 µl (REF)*

5 Open safe-lock tube and transfer 150 ml of sample in RLT to a well in the 96-well plate.
** When processing 48 or fewer samples assemble the samples in alternating columns (leaving an empty column between sample columns). This minimizes the risk of contamination and facilitates the process.*

6 Return the tube with the remaining sample to the original box and carefully record the position of each sample in the 96-well plate

** This process is best performed by teams of two with one person transferring the samples and the second recording the positions*

Silane Bead preparation (performed during swab sample thawing)

4m 45s

7 Silane Beads (stored at 4C) are washed and resuspended according to the following method (performed outside the flow hood)

7.1 Aliquot 15 µl of beads per sample into a 2 mL ED tube (calculate a 15% overage, e.g. 15 extra reactions per each 96 samples: 111×15 µl = 1665 µl of Silane Beads)

7.2 Concentrate beads to the tube wall using a DynaMar-2 magnet; remove bead storage suspension media

7.3 Wash 1X with 1 mL of RLT-plus buffer, and remove as in the previous step

7.4 Resuspend in 10 µl of RLT-plus buffer per sample

8 Pipette the required amount of pre-washed Silane Beads (10 µl/sample) into a 15 mL tube (calculate a 15% overage, e.g. 15 extra reactions per each 96 samples: 111×10 µl = 1110 µlof pre-washed Silane beads)

9 Add 70 ul 100% EtOH per sample (7770 µl for 96 samples), vortex and keep at room temperature

9.1

	Reagent	x1 sample	x96 samples (+15 of excess)
	Pre-washed Silane Beads	10 µl	1110 µl
	100 % EtOH	70 µl	7770 µl

	Total	80 µl	8880 µl

Silane bead-mediated RNA purification

20m

10 Vortex the Silane Bead-Ethanol mixture and pour it into a reservoir.

11 Use a multichannel pipette to add 80 µl of the Silane bead/ethanol into each sample well and mix well by pipetting up and down 15 times.

12 Incubate for 5 minutes at room temperature.

13 Move the plate onto a DynaMag-96 side skirted magnet and incubate for 3 minutes at room temperature.

14 Once beads are strongly magnetized and with the plate still on the magnet, aspirate the supernatant with an 8-channel vacuum aspirator.

** Alternatively use a multichannel pipette*

15 With the plate on the magnet, use the multichannel pipette to add 200 ul of 80% EtOH from another reservoir and incubate for 30 seconds.

** Do not re-supend the beads after adding the ethanol*

16 Aspirate the Ethanol with an 8-channel vacuum aspirator .

** Alternatively use a multichannel pipette*

17 Repeat the addition of 150 ul of 80% EtOH and incubate for 30 seconds.

** Do not re-supend the beads after adding the Ethanol*

18 Slowly aspirate the Ethanol with an 8-channel vacuum aspirator.

**It is very important to avoid leaving small droplets of Ethanol 80% that may inhibit the subsequent RT-qPCR step.*

19 Take out the plate from the magnet and let the beads dry inside the hood for 1 minute.

** Avoid over drying the beads of they will become sticky and difficult to re-suspend.*

** Step should be optimized in each facility as barometric pressure and relative humidity effect drying time.*

20 Add 30-50 μl of preheated 10 mM Tris pH 7.5 (from a reservoir) and resuspend the beads by pipetting up and down thoroughly.

** If samples become difficult to resuspend, incubate for 3 minutes at 37C and then try to resuspend the beads.*

21 Incubate at 37C for 3 minutes to increase the yield of the elution.

** This step may be skipped if yield is sufficient without it.*

22 Magnetize the plate again by placing it onto the DynaMag-96 side skirted magnet for 1 minute.

23 Transfer supernatant into a new 96-well plate.

24 Eluted RNA is ready for the RT-qPCR reaction.