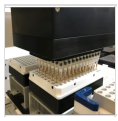

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Sars-CoV2 RNA purification with homemade SPRI beads for RT-qPCR test

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

Coronavirus Method Development Community

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ABSTRACT

The current SARS-CoV2 epidemic calls for large scale viral tests. The current testing procedure for the presence of virions involves several steps - pharyngeal/nasal swab, cell/viral lysis, RNA extraction, and an rtPCR assay targeting the viral genome and a human gene as an internal sample control. Improvement of any of these steps in terms of cost, processing time, or reagent availability, could result in a significant increase of testing capacity world-wide.

Here, we describe a rapid and efficient home made SPRI-based RNA extraction method from a lysed sample. Our approach can be fully automated, is quick (<30 min for 96 samples) and cheap (<1$ per sample), and was tested successfully on more than 250 clinical samples with approved rt-qPCR detection kits.

ATTACHMENTS

robotic_COVID_SPRI.pdf

COVID-19_SPRI_080420.zip

GUIDELINES

Note that different sources of viral samples arrive in different buffers.

This protocol was tested on samples derived by a swab into a standard Viral Transport Medium collection tube, and then mixed 1:1 with Zymo RNA/DNA shield for lysis.

This is a robot-compatible protocol. See attached zipped folder containing the robotic script and worktable for running the protocol on an EVO / Evoware platform and a pdf describing the robot configration and a human-readable protocol.

The protocol is designed to be adaptable to any robotic platform with a 96 channel arm and a manipulator arm capable of manipulating the plate containing the magnetic beads.

MATERIALS

NAME	CATALOG #	VENDOR
NaCl	53014	Sigma Aldrich
PEG 8000 Powder (Polyethylene Glycol), 500gm	V3011	Promega
Trisodium citrate dihydrate	S1804	Sigma-aldrich
Water, nuclease free		

Citation: Ayelet Rahat, Miriam Adam, Uri Shabi, Moshe Cohen, Daniel Kitsberg, Malka Nissim-Rafinia, Hagit Turm, Agnes Klochendler, Daphna Joseph-Strauss, Israa Sharkia, Matan Lotem, Gavriel Fialkoff, Ronen Sadeh, Alon Chappleboim, Yuval Dor, Nir Friedman, Dana Wolf, Naomi Habib (04/16/2020). Sars-CoV2 RNA purification with homemade SPRI beads for RT-qPCR test. https://dx.doi.org/10.17504/protocols.io.beswjefe

NAME	CATALOG #	VENDOR
1M Tris pH 7.5		Sigma
Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL	65152105050250	Ge Healthcare
Tween 20	P1379	Sigma
Ethanol		
Lithium chloride	793620	Sigma Aldrich
HCl	View	
Ethylenediaminetetraacetic acid (EDTA)	EDS	Sigma Aldrich
Lithium dodecyl sulfate	L4632	Sigma Aldrich
1M DL-Dithiothreitol solution (DTT)	646563	Sigma Aldrich
1M Tris pH 8.0	T2694	Sigma Aldrich

MATERIALS TEXT

Lysis/binding buffer (similar to Invitrogen #A33562):
100mM Tris-HCl, pH 7.5
500mM LiCl
10mM EDTA
1% LiDS (also tested with 0.5% Triton x-100)
5mM DTT

Equipment needed:
Magnet for PCR strips or 96 well plate (DynaMag-96 Side Skirted Magnet #12027 from Invitrogen)

Equipment needed when processing by hand:
PCR strips or 96 well plate (minimum volume 150 µl)
Multichannel pipette (20ul and 200ul)

The robotic platform used for the protocol:
Tecan EVO150
MCA96 arm with disposable tips
RoMa with centric fingers
Inheco robotic shaker

SAFETY WARNINGS

A risk assessment should be made when working with potenetially infectious specimen. Be sure that samples were properly handled and deactivated by certified personnal. Consult your local bio-safety staff.

BEFORE STARTING

- To prepare a large batch of home-made SPRI beads from the Sera-Mag™ Magnetic SpeedBeads follow our protocol - http://dx.doi.org/10.17504/protocols.io.bes2jege
- Take out SPRI beads from 4c storage for 30 minutes to arrive at room temperature
- Prepare fresh 80% EtOH (250 µl / sample)
- Verify you have enough Lysis/binding buffer (equal to the total volume of samples)

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SPRI viral RNA Cleanup 30m

1 **Sample dilution** 1m

Start with clinical samples collceted in virtal transport media and inactivated by dilution in lysis buffer. Add equal volume lysis/binding buffer to samples, e.g. for 28 µl, add 28 µl lysis/binding buffer

The maximal volume in the process is x3.6 the sample volume, so use the maximum amount of sample that still allows efficient robotic pipettation, depending on your setup

2 **Bead Binding** 15m

1. Add x0.8 volume SPRI beads to each diluted sample (e.g. 45 µl beads to 56 µl diluted sample)
2. Mix by pipetting x10 times, **avoid aeration during mixing to avoid foaming**
3. Incubate **00:10:00** at **25 °C** , periodically mix by pippeting
4. **Magnetize until solution clears completely** (~ **00:05:00**) to make sure you do not loose beads
5. Aspirate the supernatant slowly to avoid disturbing the magnetized beads and discard

If beads can be seen in the tips after aspirating the supernatant or if beads are found in the waste consider the following reasons:

1. Insufficient magnetization - Increase magnetization duration. The volume and buffer type affect the required duration.
2. Bead scraping by the tip - Having the tip touch or scrape the beads during movement due to misalignment. A thinner tip can increase tolerance and help avoid this issue.
3. Tip blockage and pressure buildup - If some of the tips press against the bottom of the well it will cause blockage and a buildup of negative pressure inside the tip. Once the tip moves up the pressure will cause a vortex inside the well which can disturb the beads. Have the tip at least 0.2mm above the bottom of the well and aspirate slowly

3 **80% EtOH Wash** 10m

1. Add **120 µl** of 80% EtOH
2. Move the plate from one side of the magnet to the other to immerse the beads in the wash solution
3. Wait for **00:00:30**
4. Remove the wash solution while the plate is on the magnet without disturbing the beads
5. Repeat 80% EtOH wash (steps 3.1 - 3.4)
6. Perform an extra aspiration of the residual EtOH with a fine tip
7. Remove the plate from the magnet and air dry the beads to remove traces of EtOH (~ **00:02:30** minutes at **30 °C** , until beads are dry by eye inspection). **Avoid overdrying the beads.**

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4m

4 Elute

1. Add 10mM Tris pH 8 (for 56ul beads **20 µl**) at **10 °C** - **22 °C**
2. Resuspend the beads fully in the elution buffer by pipetting
3. Incubate for **00:02:00** to allow full RNA elution
4. Place the plate on the magnet and wait for the solution to clear (~ **00:01:00**)
5. Keep on magnet, and transfer the supernatant to a new plate without disturbing the beads

To resuspend the beads in the robotic system, we alternate between several steps:

1. Wash the beads from the sides of the well - aspirate the elution liquid from the bottom of the well and dispense on the sides of the well above the z-line of the beads
2. Shaking the plate
3. Mixing by up-down pipetting about 0.5mm above the bottom of the well

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