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Viral RNA extraction low-cost protocol optimized for SARS-Cov2 at AGROSAVIA

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1 Works for me dx.doi.org/10.17504/protocols.io.bggvjtw6

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ABSTRACT

Here we present the second version of an RNA extraction protocol for SARs-cov2 using magnetic beads. This protocol was optimized in AGROSAVIA laboratories and is presented as an alternative to the limitations of commercial viral RNA extraction kits. The viral RNA extraction protocol was adapted from the protocol entitled “SARS-CoV-2 RNA purification from nasal / throat swabs collected in Viral Transfer Media” at https://bomb.bio/ and which is based on methods previously reported by the same group (Oberacker et al 2019). The optimized version here achieves Ct results like the commercial kits and reduces the cost of RNA extraction per sample by 90%, that is, 2000 Colombian pesos or 50 cents per sample. We share this protocol in the hope that it can ensure the sustainability of the diagnosis in Colombia and other countries where it is difficult and expensive to import commercial kits.

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EXTERNAL LINK

http://10.13140/RG.2.2.34818.50881

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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ATTACHMENTS

English translation of_ Protocolo de extracción de ARN viral optimizado para SARS-Cov2.docx

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GUIDELINES

Here we present the second version of an RNA extraction protocol for SARs-cov2 using magnetic beads. This protocol was optimized in AGROSAVIA laboratories and is presented as an alternative to the limitations of commercial viral RNA extraction kits. The viral RNA extraction protocol was adapted from the protocol entitled "SARS-CoV-2 RNA purification from nasal / throat swabs collected in Viral Transfer Media" at https://bomb.bio/ and which is based on methods previously reported by the same group (Oberacker et al 2019). The optimized version here achieves Ct results like the commercial kits and reduces the cost of RNA extraction per sample by 90%, that is, 2000 Colombian pesos or 50 cents per sample. We share this protocol in the hope that it can ensure the sustainability of the diagnosis in Colombia and other countries where it is difficult and expensive to import commercial kits.

MATERIALS TEXT

Reagent List

Reagent
Guanidine Thiocyanate (GITC)
Sarkosyl
Magnetic beas GE Healthcare Sera-Mag Carboxylate-Modified
Isopropanol
EDTA
Ethanol
Proteinase K (20 mg/mL)

Preparation of reagents

3.1 Paramagnetic beads

a. Take 1 ml of Sera-Mag pearls, be sure to mix well before you start.

b. Wash the beads 3 times in 1 ml of TE buffer (this is to remove sodium azide from the solution), use the magnet to separate the beads from the washes, remove the TE.

c. Re-suspend in 50 ml of TE buffer.

3.2 GITC Lysis Buffer

a . 35.46 g of GITC.

b. 2.5 mL of 1 M Tris HCl pH 7.6-8.0 stock solution

c. 1 g of Sarkosyl.

d. 2 ml of 0.5 M EDTA stock solution.

e. Fill to 50 ml with sterile distilled water.

Reagent prices

The price of the reagents was quoted with national suppliers and the average prices for the commercial houses are presented.

Table 1. Reagents and prices

Reagent	**Quantity**	**Approximate Price * (pesos)/USD**	**Number of samples**	**Price per sample * (pesos)/USD**
Guanidine Thiocyanate (GITC)	500 g	$2 000 000/500	70oo	$286/0.07
Sarkosyl	25 g	$ 1 470 000/367.50	12500	$117/0.02
Magnetic beas GE Healthcare Sera-Mag Carboxylate-Modified	15 mL	$3 000 000/750	18750	$160/0.04
Isopropanol	500 mL	$300 000/75	1250	$240/0.06
EDTA	250 g	$ 250 000/62.5	1000	$250/0.06
Ethanol	2500 mL	$200 000/50	6250	$ 32/0.008

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Proteinase K (20 mg/mL)	6 mL	300 000/75	500	$600/0.15
			Total	$1685/0.45 (pesos/USD)

* price in Colombian pesos

BEFORE STARTING

The adjusted protocol presented here is susceptible to other improvements such as those reported for the detection of respiratory viruses (He et. al 2017). 2. Similar protocols and how to build the magnetic racks can be found on the page of the original Authors of the method (https://bomb.bio/). 3. We are not responsible for any accident, infection, or legal implication resulting from the use of protocols adjusted here or hosted at BOMB.bio.

1 Pre-processing of mucous samples.

1.1 Dilute **100 µl** of the sample in **300 µl** of Saline Solution [**0.8 Mass / % volume** , vortex mix **00:00:10** . Use only **100 µl** of this dilution to star protocol. In step 2.3 add **540 µl** isopropanol after incubation.

2 Sample Processing (in 1.5 ml tubes)

2.1 To **100 µl** of GITC buffer add **12.5 µl** of Proteinase K **20 mg/ml**], **200 µl** of the sample and mix by vortex for **00:00:10** .

2.2 Incubate the sample for **00:10:00** at **60 °C** , vortexing every **00:02:00** .

2.3 Place the tube in a rack and add **270 µl** of Isopropanol and **40 µl** of magnetic beads (see preparation below). Mix by inverting 8 times.

2.4 Place the tube (s) in the magnetic rack for **00:10:00** without moving.

2.5 Without moving the tube from the magnetic rack, withdraw approximately **610 µl** of the solution

2.6 Without moving the tube from the magnetic rack, add **150 µl** of Isopropanol and wait 30 seconds and remove the isopropanol from the tube.

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2.7 Without moving the tube from the magnetic rack, add **200 µl** of **70 % (v/v)** Ethanol, and remove from the tube.

2.8 Without moving the tube from the magnetic rack, repeat previous washing 2.7 and extract the Ethanol to the maximum.

2.9 Without moving the tube from the magnetic rack, allow drying for approximately **00:05:00** .

2.10 Add **30 µl** l of molecular grade water to the tube trying to discharge the liquid on top of the tube walls where the beads are located.

2.11 Remove the tube from the magnetic rack and vortex **00:00:10** to allow the beads to enter the solution and incubate **00:01:00** at room temperature in a normal rack

2.12 Place the tube back in the magnetic rack and for **00:03:00** to **00:05:00** , and transfer **20 µl** of the bead-free elution to a new tube and store at **-80 °C** .

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