RH-a-1

Automated_Viral RNA Extraction from Pharyngeal Swab with SARS-CoV-2

Protocol

2 ml microtube※1

← Lysis Buffer(LRV) : 200 µl

← 1 mg/mL Carrier RNA*2 : 20 µl※3

← Pharyngeal Swab : 200 µl

Vortex (maximum speed) : 15 sec

Flash spin down

Incubation : 10 min, RT

Set into the device

Protocol: RNA VIRUS
(Elution volume : 50 µl)

*Please refer to Quick Start Guide or operation manual to know how to set sample tube.

1. Add 185 µl of Solubilization Buffer (SRV)
2. Mix by pipetting
3. Add 185 µl of Ethanol(>99%)
4. Mix by pipetting
5. Apply the lysate into the cartridge
6. Pressurizing
7. Wash 3 times by Wash Buffer (WRV)
8. Add selected volume of Elution buffer and elute viral RNA into collection tube.

Viral RNA

*1 Following microtube are recommended.
#BM4020
(BM instrument co., ltd)
#72.695.700,
#72.695.500S
(SARSTEDT)

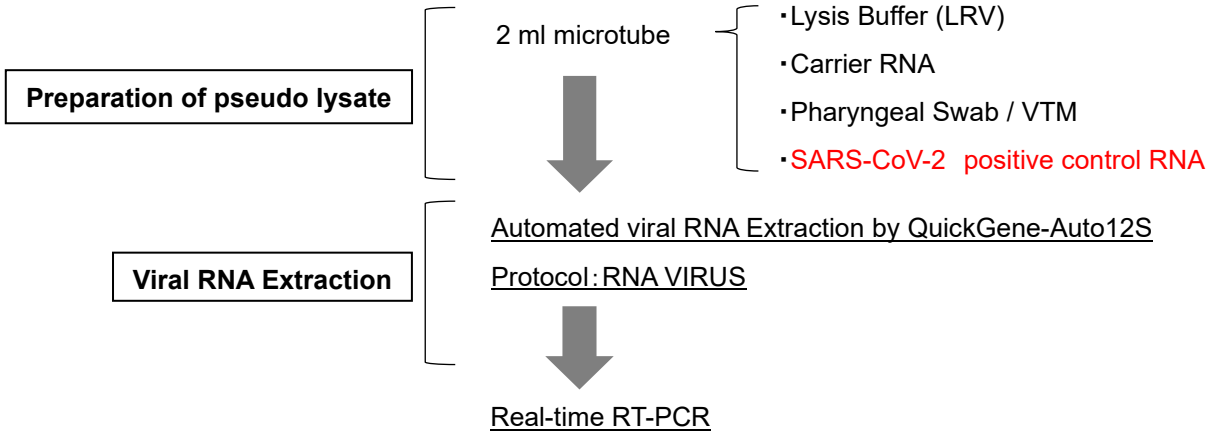
*2 Following Carrier RNA is recommended.
#4382878: Carrier RNA
(Thermo Fisher Scientific Inc Applied Biosystems™)

*3 20 µg of Carrier RNA is recommended per sample.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Materials & methods

Experimental flow chart



Preparation of pseudo lysate

- Materials
  - ○ VTM： # SGVTM-3R Viral Transport Media (SUGIYAMA-GEN Co. LTD)
  - ○ Specimen Collection Swab： #25-806 1WC EC Cap-Shure 6" Sterile Standard Cotton Swab & Protective Cap w/ Wooden Handle (Puritan Medical Products Co. LLC)
  - ○ SARS-CoV-2 positive control RNA： #954519 Coronavirus 2019 (COVID-19) RNA Control (RUO) (Thermo Fisher Scientific Inc. AcroMetrix™)
    RNA Control（954519）
  - ○ Carrier RNA： #4382878 Carrier RNA(Thermo Fisher Scientific Inc. Applied Biosystems™)

1. Prepare the Viral Transport Media (3ml / 15 ml tube).
2. Scrape the pharynx with a specimen collection swab and add it to the VTM (Below, pharyngeal swab).
3. Artificially add SARS-CoV-2 positive control RNA to the pharyngeal swab.

Viral RNA Extraction

Automatically extract viral RNA from 200 µl of lysate (containing pharyngeal swab, SARS-CoV-2 positive control RNA, and Carrier RNA 20 µg) using AS-RV kit and QuickGene-Auto12S (Elution:50 µl).

Sample	SARS-CoV-2 positive control RNA	No.
Pharyngeal swab / VTM ＋ SARS-CoV-2 positive control RNA	100 copies / lysate(200 µL)	K1
	500 copies / lysate(200 µL)	K2
Negative Control Pharyngeal swab / VTM	–	NC

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Real-time RT-PCR

Detect Viral RNA by Real-time RT-PCR using 10 µL of extracted RNA and SARS-CoV-2 detection kit.

- SARS-CoV-2 detection kit
  # RC300A SARS-CoV-2 Direct Detection RT-qPCR Kit(Takara Bio Inc.) [Positive：Ct＜40 cycle]
- Real-time RT-PCR equipment
  QuantStudio® 5 Real-Time PCR System(Thermo Fisher Scientific Inc.)

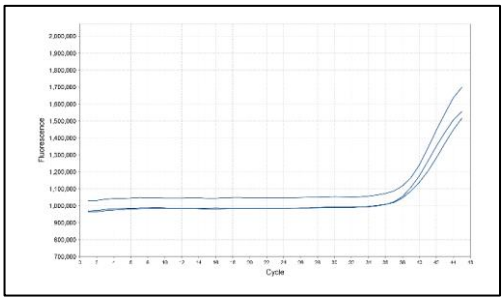
Results

Real-time RT-PCR：K1, K2, NC

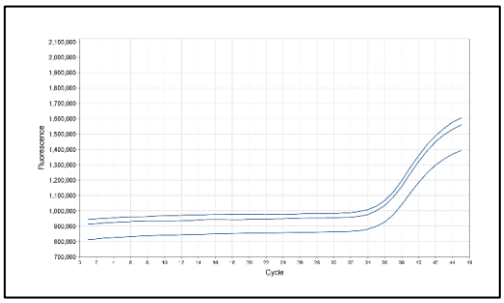
✓ **Ct (Threshold Cycle)**

	100 copies / lysate	500 copies / lysate	Negative Control	PCR Positive control (1×10⁵ copies/well)
No.	**K1**	**K2**	**NC**	**PC**
Ct (cycle)	**36.6**	**34.6**	**Undetected**	**23.7**

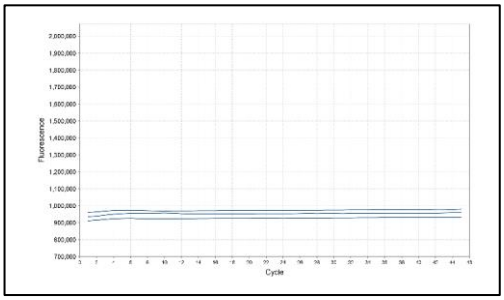
✓ **Amplification Plot**



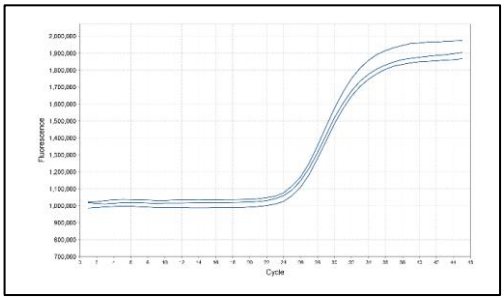
K1（100 copies / lysate）



K2（500 copies / lysate）



Negative Control



PCR Positive control

✓ We have verified that QuickGene-Auto12S and AS-RV kit can extract viral RNA from lysates containing pharyngeal swabs and SARS-CoV-2 positive control RNA (100 and 500 copies).

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