

X.Total RNA Extraction from Viruses



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LRT, SRT, and WRT refer to reagents contained in the QuickGene RNA Extraction kit.

Reagents:

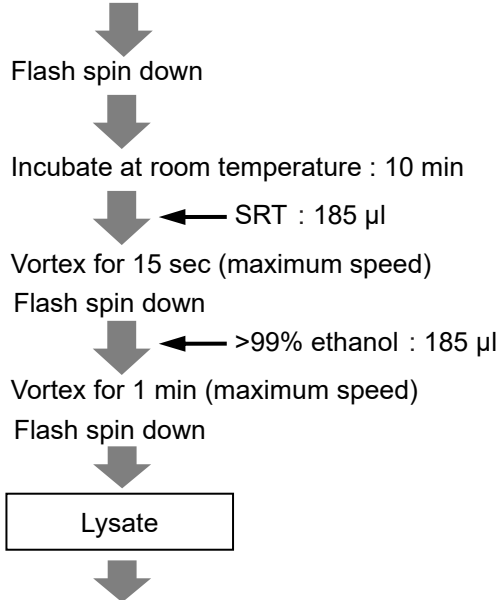
- Lysis Buffer (LRT),
- Solubilization Buffer (SRT)
- Wash Buffer (WRT)
- Elution Buffer (CRT)

RH-X1

Viral RNA Extraction from Serum

Protocol

Vortex for 30 sec (maximum speed), adding 10 μ l of 10mg/ml Carrier RNA ^{*1} solution and 150 μ l of test serum to 200 μ l of LRT (TCEP added) ^{*2}.



Set into the device:

- QG-Mini480 or QG-Mini80^{*a}
- QG-Auto12S or QG-Auto24S^{*b}

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

1. Apply the lysate into the cartridge
2. Pressurizing
3. Wash 1 time by Wash Buffer (WRT^{*4})
4. DNase treatment (if needed)
5. Wash 2 times by Wash Buffer (WRT^{*4})
6. Add selected volume of Elution buffer (Elution volume : 100 μ l) ^{*3} and elute total RNA into collection tube.

Total RNA

The following items are included in the extraction kit:
Lysis Buffer (LRT),
Solubilization Buffer (SRT),
Wash Buffer (WRT),
Elution Buffer (CRT).

*1 Carrier RNA., which is added for prevention of virus RNA decomposition by RNase in sample and also nonspecific adsorption of a small amount of refined RNA.

*2 Add 20 μ l of 0.5mol/L TCEP per 1 ml of LRT.

Company: FUJIFILM Wako
Pure Chemical Corporation
Name: 0.5mol/L TCEP Solution
Catalog No. : 207-20151

*3 The volume of the from each cartridge is 100 μ l. The volume of CRT can be reduced to 50 μ l, but in that case, elution efficiency might be decreased.

*4 Please use ethanol added Wash Buffer (WRT)

*a QuickGene RNA tissue kit S II (RT-S2) kit is used for QG-Mini480 or QG-Mini80

*b QuickGene AutoS RNA Tissue kit (AS-RT) kit is used for QG-Auto12S or QG-Auto24S

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).

RH-X4

Virus RNA Extraction from sputum

Protocol

Collect sputum samples (100µl) in a 1.5ml microtube

↓
Add 50µl of PBS or saline,
and 10 µl of 10mg/ml Carrier RNA *1 solution

↓
Vortex (Maximum speed) : 60 sec.

↓ ← Add LRT (TCEP added) *2 : 200 µl
Incubate at room temperature : 10 min

↓
Vortex (Maximum speed) : 15 sec

Flash spin down

↓ ← SRT : 175 µl

Vortex (Maximum speed) : 15 sec

Flash spin down

↓ ← >99% ethanol : 175 µl

Vortex (Maximum speed) : 1 min

Flash spin down

Lysate

Set into the device:

- QG-Mini480 or QG-Mini80^{*a}
- QG-Auto12S or QG-Auto24S^{*b}

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

1. Apply the lysate into the cartridge
2. Pressurizing
3. Wash 1 time by Wash Buffer (WRT^{*4})
4. DNase treatment (if needed)
5. Wash 2 times by Wash Buffer (WRT^{*4})
6. Add selected volume of Elution buffer
(Elution volume : 100 µl)^{*3}
and elute total RNA into collection tube.

Total RNA

The following items are included in the extraction kit:
Lysis Buffer (LRT),
Solubilization Buffer (SRT),
Wash Buffer (WRT),
Elution Buffer (CRT).

*1 Carrier RNA., which is added for prevention of virus RNA decomposition by RNase in sample and also nonspecific adsorption of a small amount of refined RNA.

*2 Add 20 µl of 0.5mol/L TCEP per 1 ml of LRT.

Company: FUJIFILM Wako
Pure Chemical Corporation
Name: 0.5mol/L TCEP
Solution
Catalog No. : 207-20151

*3 The volume of the eluate from each cartridge is 100µl.
The volume of CRT can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

*4 Please use ethanol added Wash Buffer (WRT)

*a QuickGene RNA tissue kit S II (RT-S2) kit is used for QG-Mini480 or QG-Mini80

*b QuickGene AutoS RNA Tissue kit (AS-RT) kit is used for QG-Auto12S or QG-Auto24S

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
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