

X.Total RNA Extraction from Viruses

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LRT, SRT, and WRT refer to reagents from the QuickGene RNA Extraction kit, QG-RT-S2 and QG-AS-RT.

Reagents:

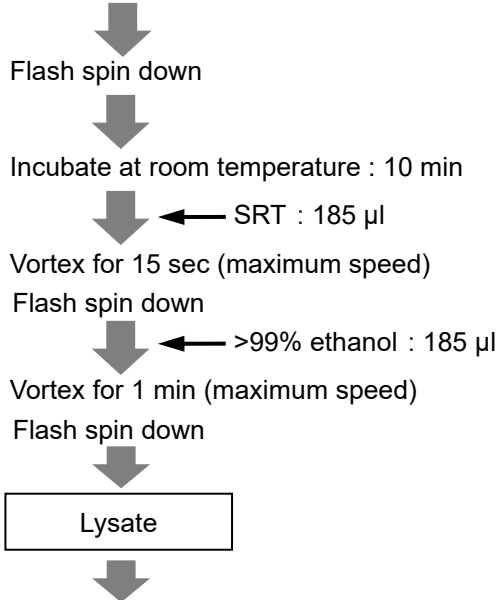
- Lysis Buffer (LRT),
- Solubilization Buffer (SRT)
- Wash Buffer (WRT)
- Elution Buffer (CRT)

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

^{*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.

The following items are included in the extraction kit:

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

^{*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

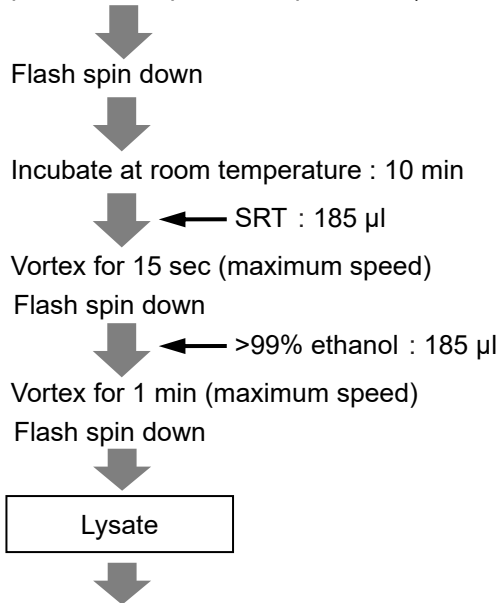
^{*b} QuickGene AutoS RNA Tissue kit (AS-RT) kit is used for QG-Auto12S or QG-Auto24S

RH-X2

Viral RNA Extraction from Bronchoalveolar lavage (endo) tracheal aspirate/ nasopharyngeal aspirate/nasal wash

Protocol

Vortex for 30 sec (maximum speed), adding 10 µl of 10mg/ml Carrier RNA *1 solution and 150 µl of test sample to 200 µl 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 (WRT⁴)
4. DNase treatment (if needed)
5. Wash 2 times by Wash Buffer (WRT⁴)
6. Add selected volume of Elution buffer
(Elution volume : 100 µl)³
and elute total RNA into collection tube.

Total RNA

*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.

The following items are included in the extraction kit:

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

*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

RH-X3

Viral RNA Extraction from Nasopharyngeal swab or /and oropharyngeal swab

Protocol

Collect swab samples in 150 μ l PBS or Saline in a 1.5ml microtube,
adding 10 μ l of 10mg/ml Carrier RNA ^{*1} solution

Vortex (Maximum speed) : 30 sec. Dispose the swab

Incubate at room temperature : 10 min

Incubate at room temperature : 10 min

Vortex (Maximum speed) : 15 sec

Flash spin down

Vortex (Maximum speed) : 15 sec

Flash spin down

Vortex (Maximum speed) : 15 sec

Flash spin down

Vortex (Maximum speed) : 1 min

Flash spin down

Flash spin down

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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 μ l)^{*3}
and elute total RNA into collection tube.

Total RNA

*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.

The following items are included in the extraction kit:

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

*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 eluate 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

*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