

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)

Viral RNA Extraction from Serum

Protocol

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

Flash spin down

Incubate at room temperature : 10 min

← SRT : 185 µl

Vortex for 15 sec (maximum speed)

Flash spin down

← >99% ethanol : 185 µl

Vortex for 1 min (maximum speed)

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.

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 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

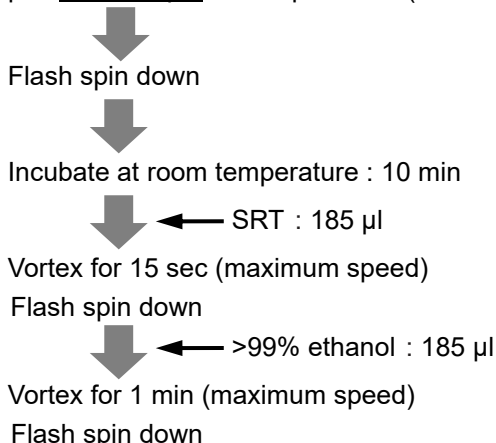
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

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}.



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.

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

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-X3

Viral RNA Extraction from Nasopharyngeal swab or /and oropharyngeal swab

Protocol

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

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

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) : 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.

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

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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

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).