



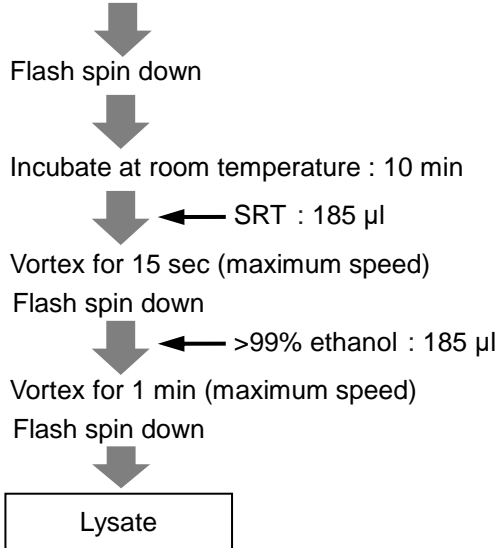
18. Total RNA Extraction from Virus

RH-1

Hepatitis C Virus (HCV) RNA Extraction from Serum

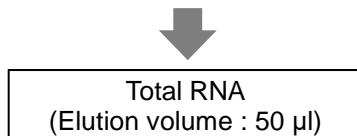
Protocol

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 (containing 2-ME) ^{*2}.



Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)



*1 Carrier RNA., which is added for prevention of virus RNA decomposition by RNase in serum and also nonspecific adsorption of a small amount of refined RNA. PolyA RNA (Sigma-Aldrich Company) was used. Company : Sigma-Aldrich Name: Polyadenylic acid potassium salt Catalog No. : P9403

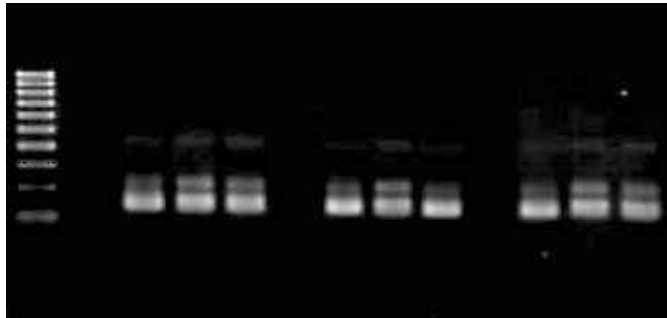
*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

Other

- Detection of HCV virus RNA by RT-PCR / nested PCR

M a b c d e f g h i j k l



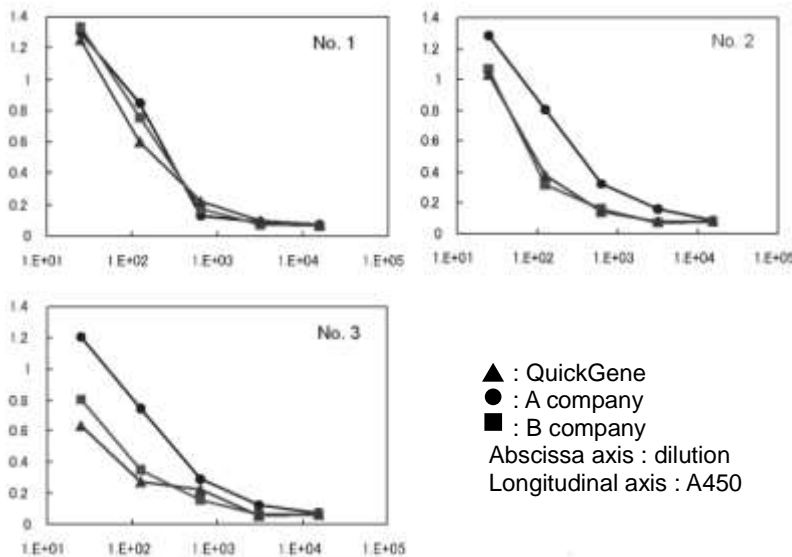
M : marker (100 bp ladder)
a, e, i : HCV negative normal human
b, f, j : HCV positive patient No.1
c, g, k : HCV positive patient No.2
d, h, l : HCV positive patient No.3

a ~ d : QuickGene
e ~ h : A company
i ~ l : B company

Hepatitis C RNA could be detected by RT-PCR / nested PCR method, using RNA prepared from serum of HCV infected patient with QuickGene.

- Detection of HCV RNA

For 3 kinds of RNA obtained with QuickGene system, A company product and B company product, detection sensitivity of HCV RNA was examined using AMPLICOR detection system (hybridization method).



In comparison with B company, low reactivity of about 1/5 at maximum was found for 2 analytes among 3 analytes. On the other hand, meaningful difference in reactivity was not found for RNA prepared with QuickGene and A company product.

Regarding this sensitivity deviation from AMPLICOR, it is considered to be one cause that small fragments of decomposed RNA do not come into samples for QuickGene and A company product.

It was shown that HCV RNA in ordinary serum of patient can be detected with adequate sensitivity by serum RNA prepared with QuickGene. In QuickGene, troublesome operations such as isopropanol precipitation and collection by centrifugation which are contained in RNA preparation protocol of AMPLICOR are unnecessary and RNA preparation becomes easy.

Common protocol is usable for the following

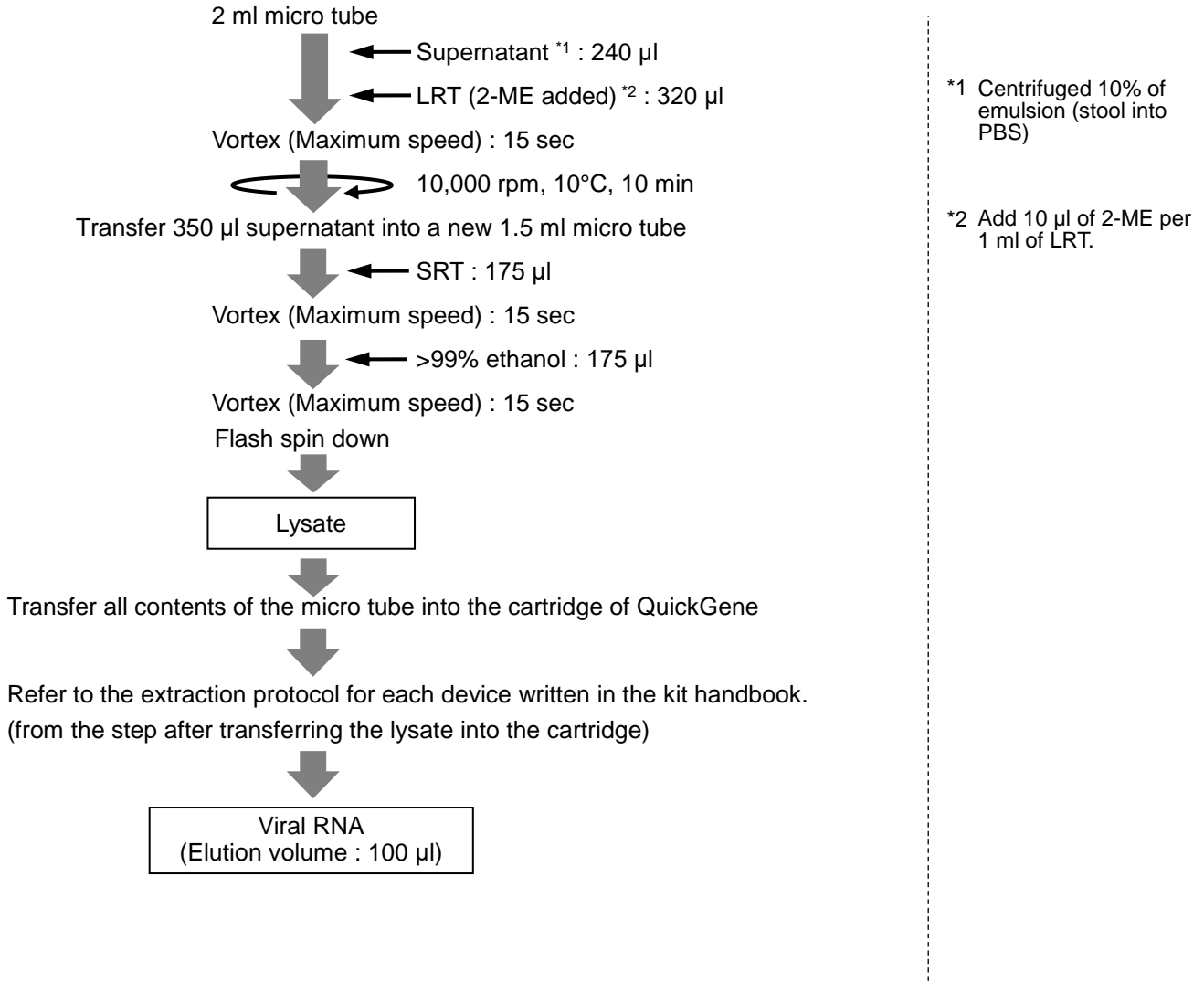
HIV

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-2

Norovirus RNA Extraction from Stool

Protocol A (PCR Method)



Results

Other

Inspection

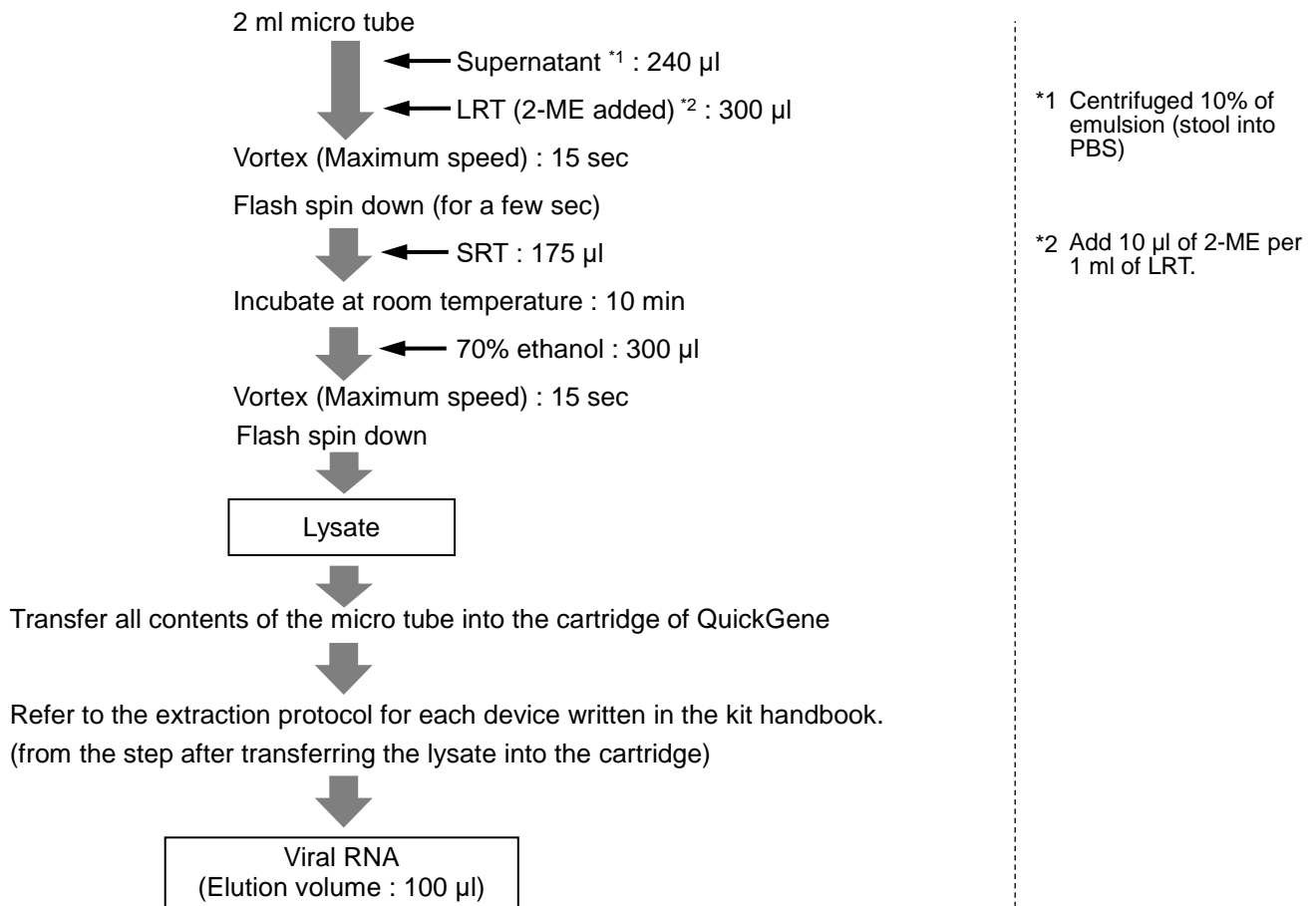
PCR Method (approved by Ministry of Health, Labour and Welfare / Pharmaceuticals and Medical Devices 2007 Nov. 5)

<http://www.mhlw.go.jp/topics/syokuchu/kanren/kanshi/031105-1.html>

Common protocol is usable for the following

No Data

Protocol B (TRC Method)



Results

Other

▪ Inspection

Norovirus inspection : Tosoh Corporation's TRCRapid-160 system
<http://www.tosoh.co.jp/science/trc/real.html>

Common protocol is usable for the following

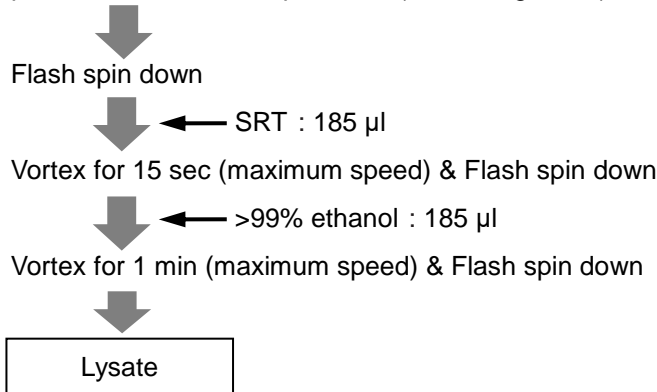
No Data

RH-3

RNA Extraction from Serum of HIV Patient and Human Serum which spiked HIV Virus Particle and Detection Limit of HIV RNA

Protocol A

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 (containing 2-ME) *2.



Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume : 50 µl)

*1 Carrier RNA., which is added for prevention of virus RNA decomposition by RNase in serum and also nonspecific adsorption of a small amount of refined RNA. PolyA RNA (Sigma-Aldrich Company) was used. Company : Sigma-Aldrich Name: Polyadenylic acid potassium salt Catalog No. : P9403

*2 Add 10 µl of 2-ME per 1 ml of LRT.

Results

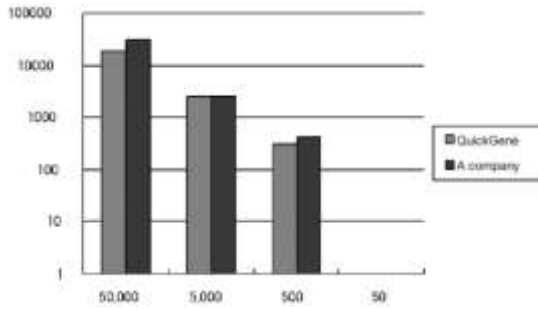
Other

- Refinement of HIV RNA from human serum which spiked HIV virus particle

Virus solution of HIV was added to normal human pool serum in concentrations in the table below. According to the above protocol, HIV RNA was detected quantitatively using AMPLICOR detection system (PCR-hybridization) for RNA prepared by use of QuickGene and RNA extracted by A company standard protocol.

| Spiked virus amount (number of virus particles/ml) | Calculated value(copy/ml) | |
|---|---------------------------|-----------|
| | QuickGene | A company |
| 50,000 | 18623.6 | 30827 |
| 5,000 | 2467 | 2471.2 |
| 500 | 304.9 | 435.4 |
| 50 | -6.6 | -2.6 |

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).



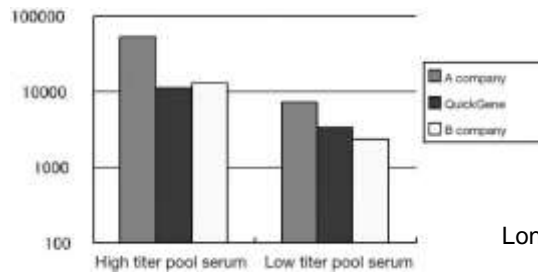
Abscissa axis : spiked virus particle amount
 Longitudinal axis : calculated HIV RNA amount

From above results, HIV RNA could be detected in detection sensitivity equivalent to A company using RNA extracted with QuickGene. The sensitivity was about several hundred virus particles/ml.

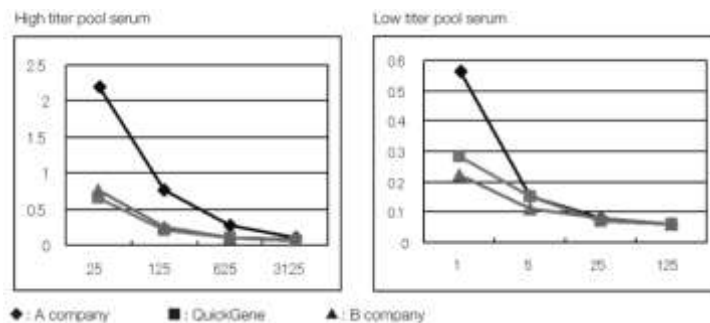
▪ Refinement of HIV RNA from human serum which spiked HIV virus particle

RNA was prepared from pool serum (2 analytes of high titer and low titer) of HIV patient, using QuickGene, B company product and A company product, and HIV RNA was detected quantitatively with AMPLICOR detection system.

| | High titer pool serum | Low titer pool serum |
|-----------|-----------------------|----------------------|
| A company | 53908.8 | 7391.2 |
| QuickGene | 11178.6 | 3348.9 |
| B company | 13157.2 | 2425.7 |



Longitudinal axis : HIV RNA amount (copy/ml)



Abscissa axis : degree of dilution of PCR
 Longitudinal axis : absorbance at 450 nm

From above results, regarding HIV RNA detection for patient serum, the strongest response was obtained for A company product. Equivalent response was obtained for QuickGene and B company product, which was about 1/2 to 1/5 of A company product. This detection method aims calculation of order of copy number, and deviations of 1/2, 1/5 can be regarded as in error range between experiments. The 3 values are in the range of same order. So, they are equivalent from the point of view of detection sensitivity. Therefore, it was shown that HIV RNA can be detected from HIV patient serum quantitatively and in high sensitivity by this protocol using QuickGene.

Common protocol is usable for the following

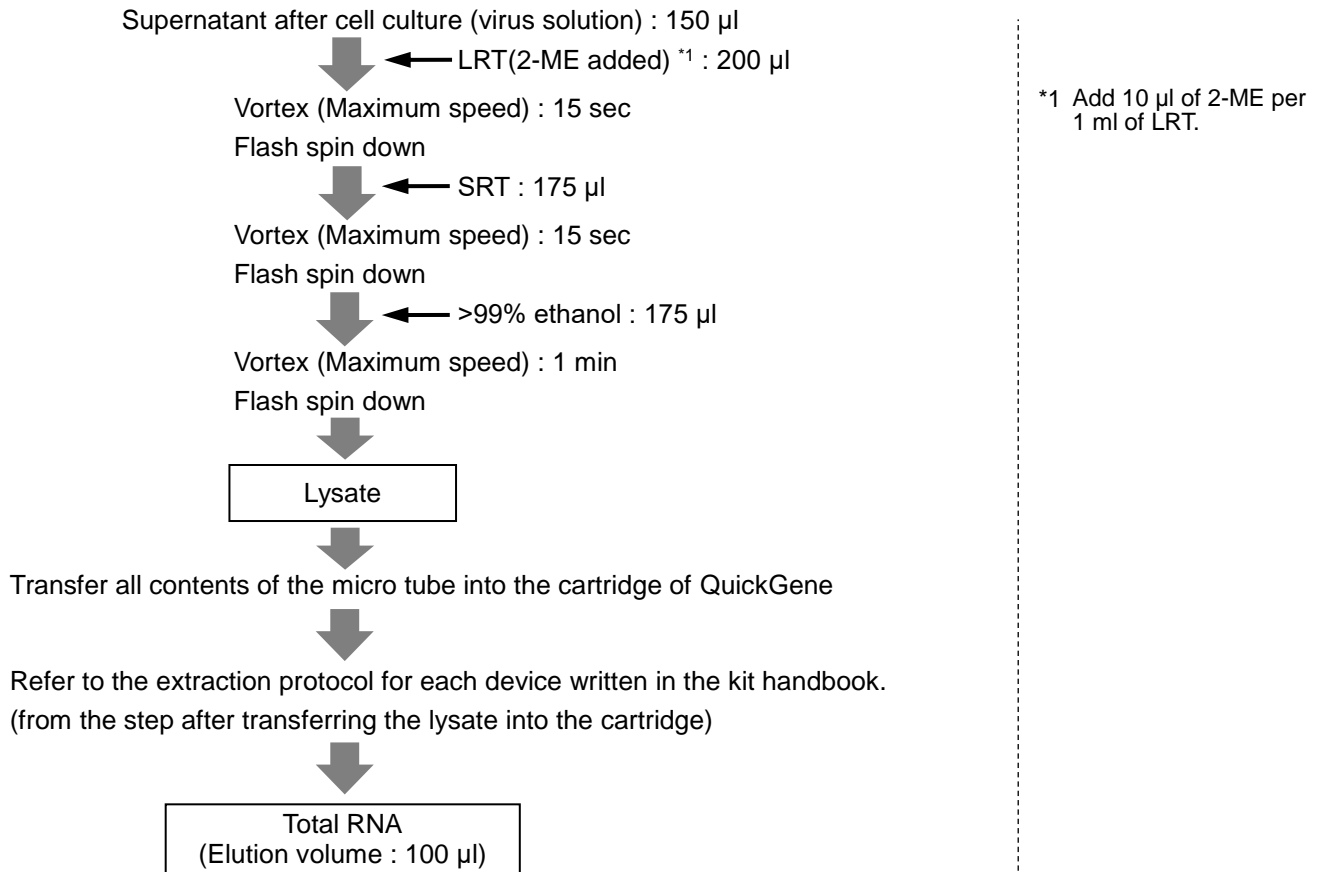
HCV

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-4

Total RNA Extraction from Influenza Virus Solution

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

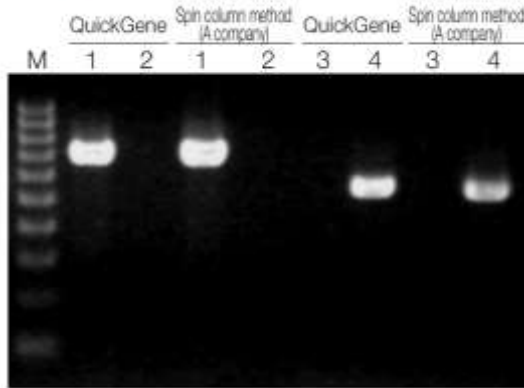
Results

Other

- RT-PCR

RT-PCR was performed with AH3 type influenza-specific primer and B type influenza-specific primer for total RNA extracted from influenza virus solution using QuickGene system and Spin column method (A company).

Confirmation of virus type selectivity



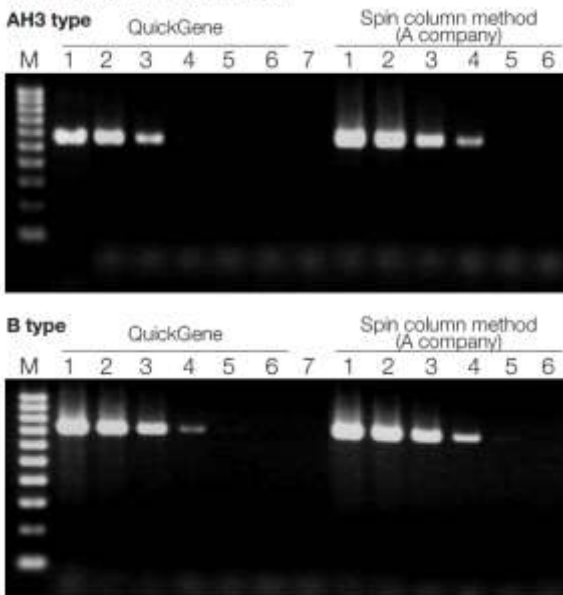
Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
 1 : AH3 type Influenza virus RNA
 2 : B type Influenza virus RNA
 3 : AH3 type Influenza virus RNA
 4 : B type Influenza virus RNA

Primer : 1,2 AH3 type influenza-specific primer
 3,4 B type influenza-specific primer

RT-PCR products were detected only with specific primer for each total RNA.

Confirmation of virus RT-PCR



Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
 1 : Influenza virus, 10⁶ pfu/ml
 2 : Influenza virus, 10⁵ pfu/ml
 3 : Influenza virus, 10⁴ pfu/ml
 4 : Influenza virus, 10³ pfu/ml
 5 : Influenza virus, 10² pfu/ml
 6 : Influenza virus, 10¹ pfu/ml
 7 : Negative control

RT-PCR products of AH3 type influenza and B type influenza were detected for each total RNA.

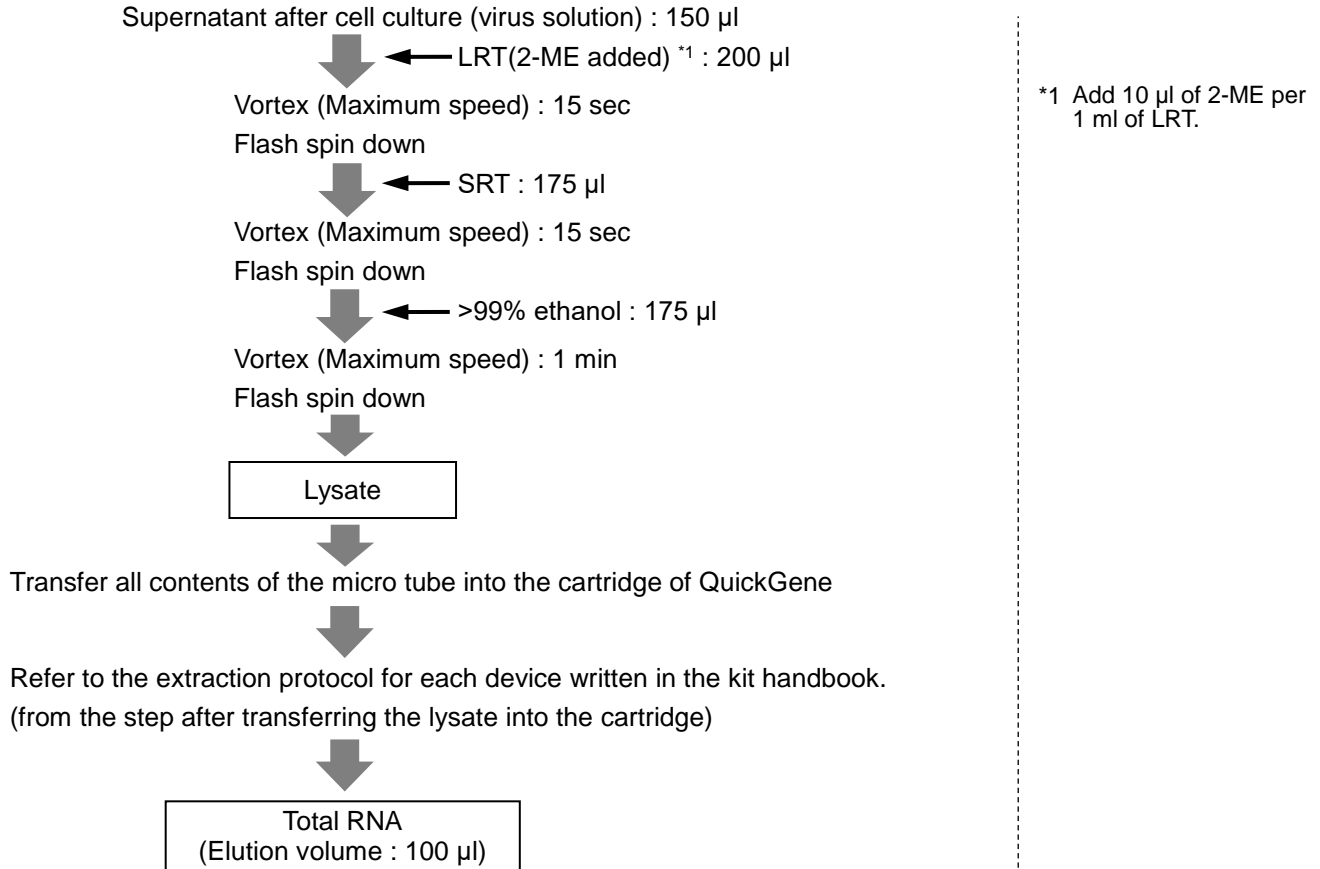
Common protocol is usable for the following

Measles Virus, Respiratory Syncytial (RS) Virus

RH-5

Total RNA Extraction from Measles Virus Solution

Protocol

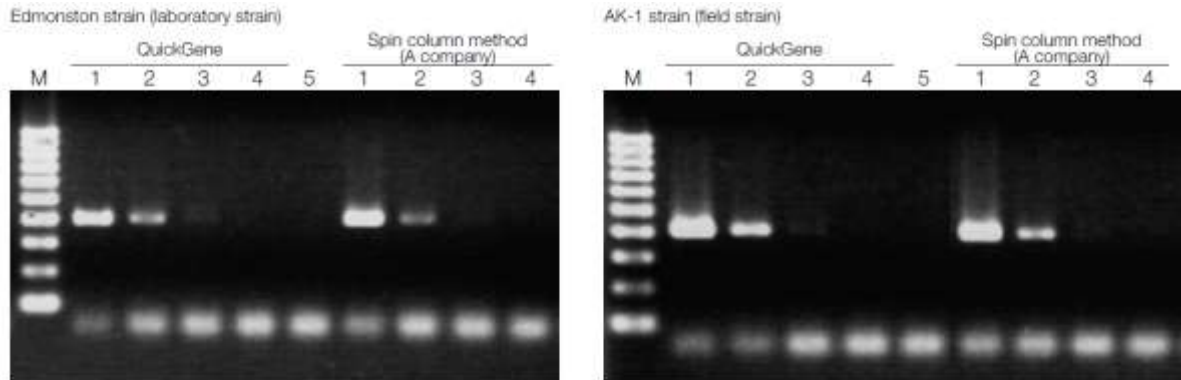


Results

Other

- RT-PCR

RT-PCR was performed with viral hemagglutinin (HA)-specific primer for total RNA extracted from measles virus solution using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2.0% agarose/1 x TAE

- M: 100 bp DNA Ladder
- 1 : Measles virus, 10^5 pfu/ml
- 2 : Measles virus, 10^4 pfu/ml
- 3 : Measles virus, 10^3 pfu/ml
- 4 : Measles virus, 10^2 pfu/ml
- 5 : Negative control

RT-PCR products of HA were detected for each total RNA.

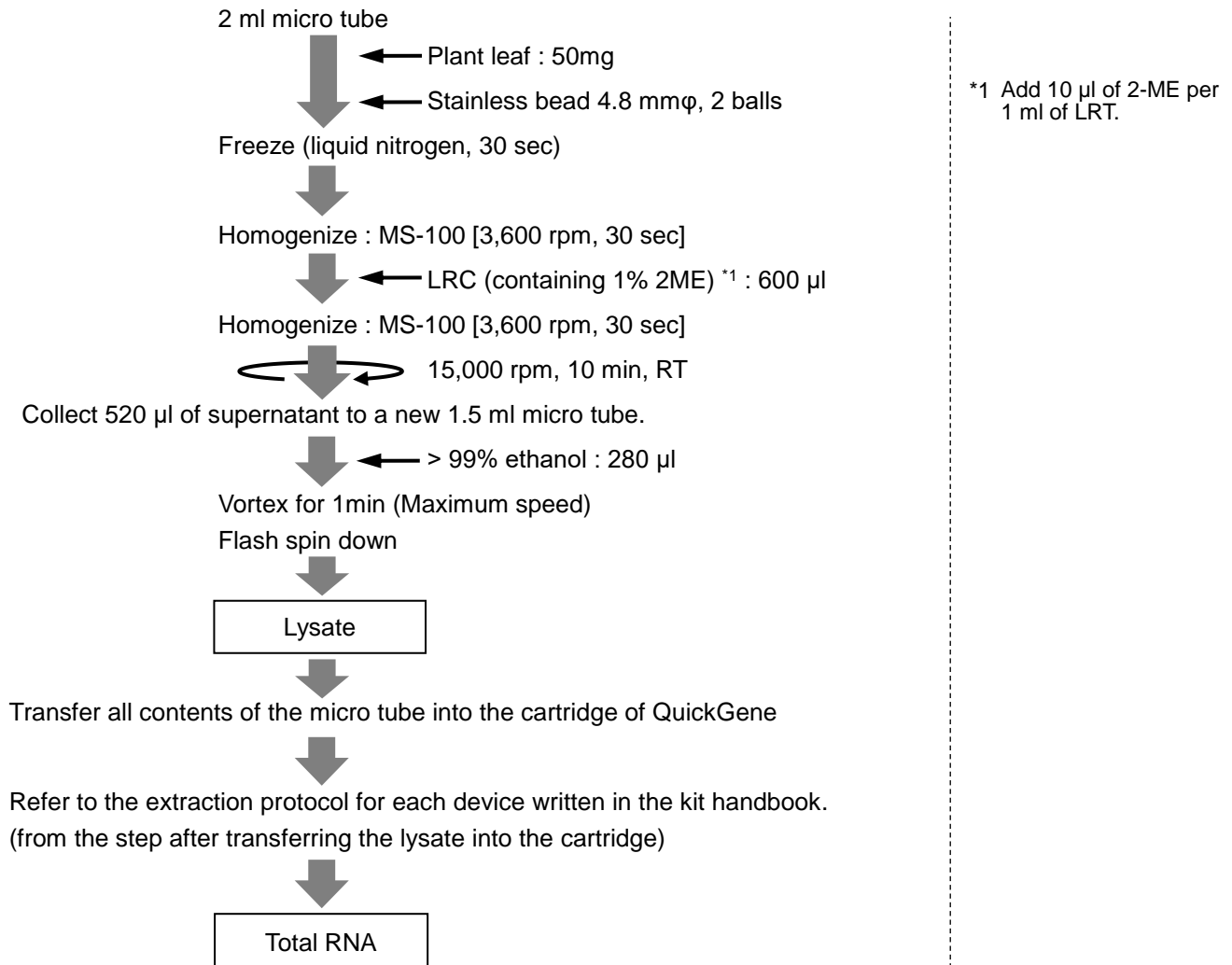
Common protocol is usable for the following

Influenza Virus, Respiratory Syncytial (RS) Virus

RH-6

Total RNA Extraction from Plant virus

Protocol



Results

No Data

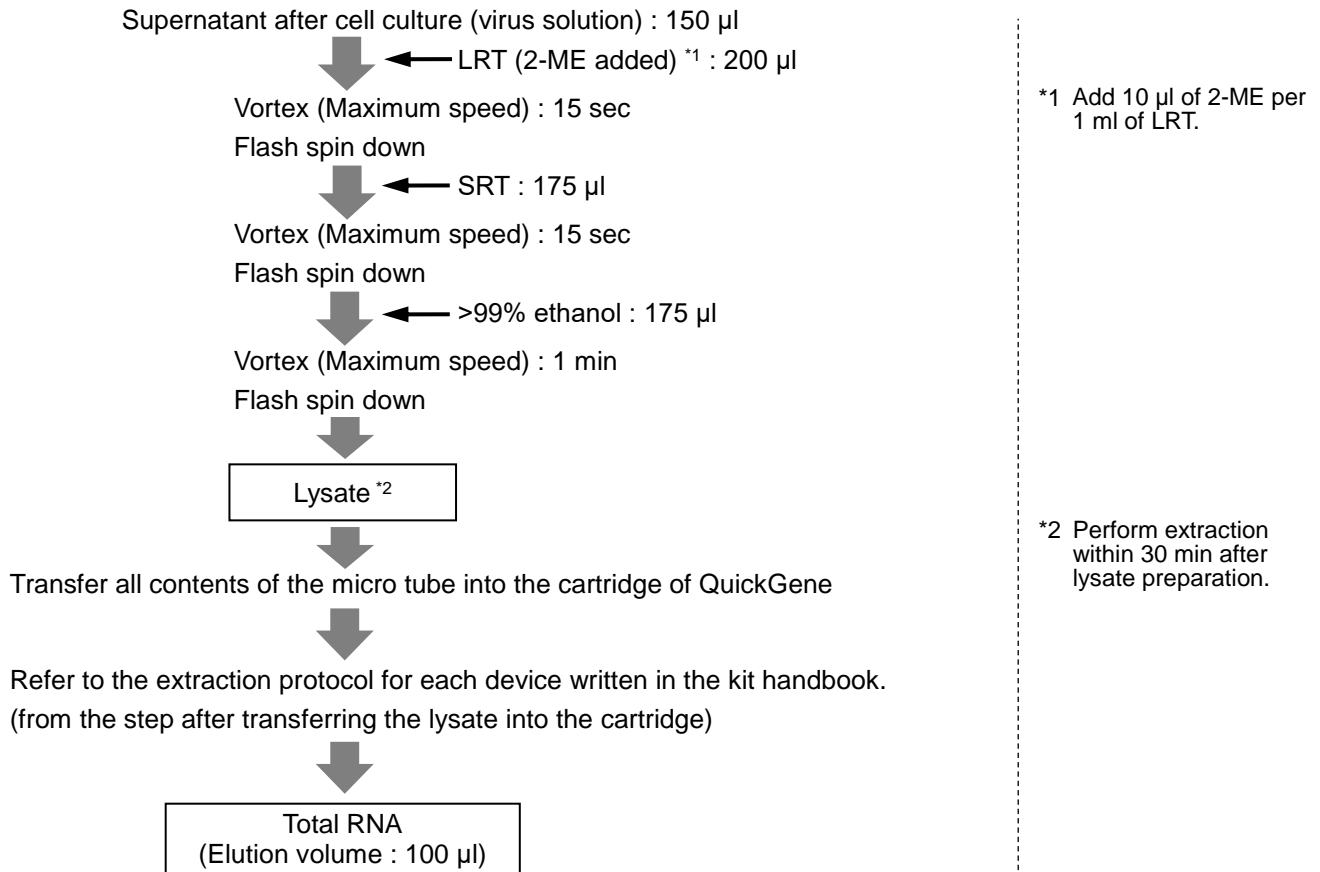
Common protocol is usable for the following

No Data

RH-7

Total RNA Extraction from Respiratory Syncytial (RS) Virus Solution

Protocol

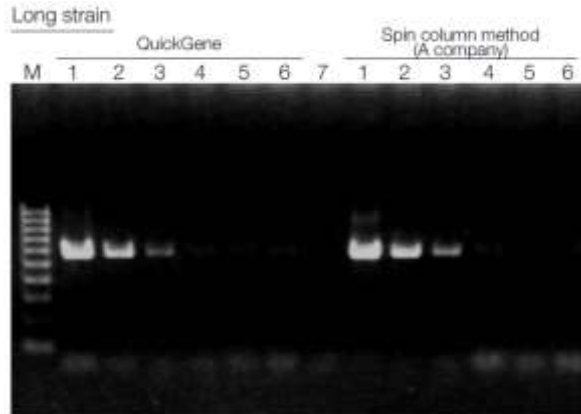


Results

Other

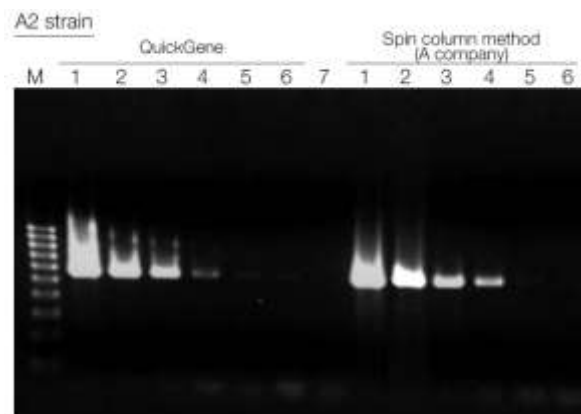
▪ RT-PCR

RT-PCR was performed with primer characteristic to G protein gene of RS virus for total RNA extracted from RS virus solution using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
 1 : RC virus, 10⁵ pfu/ml
 2 : RC virus, 10⁴ pfu/ml
 3 : RC virus, 10³ pfu/ml
 4 : RC virus, 10² pfu/ml
 5 : RC virus, 10¹ pfu/ml
 6 : RC virus, 1 pfu/ml
 7 : Negative control



RT-PCR products of G protein gene of RS virus were detected for each total RNA.

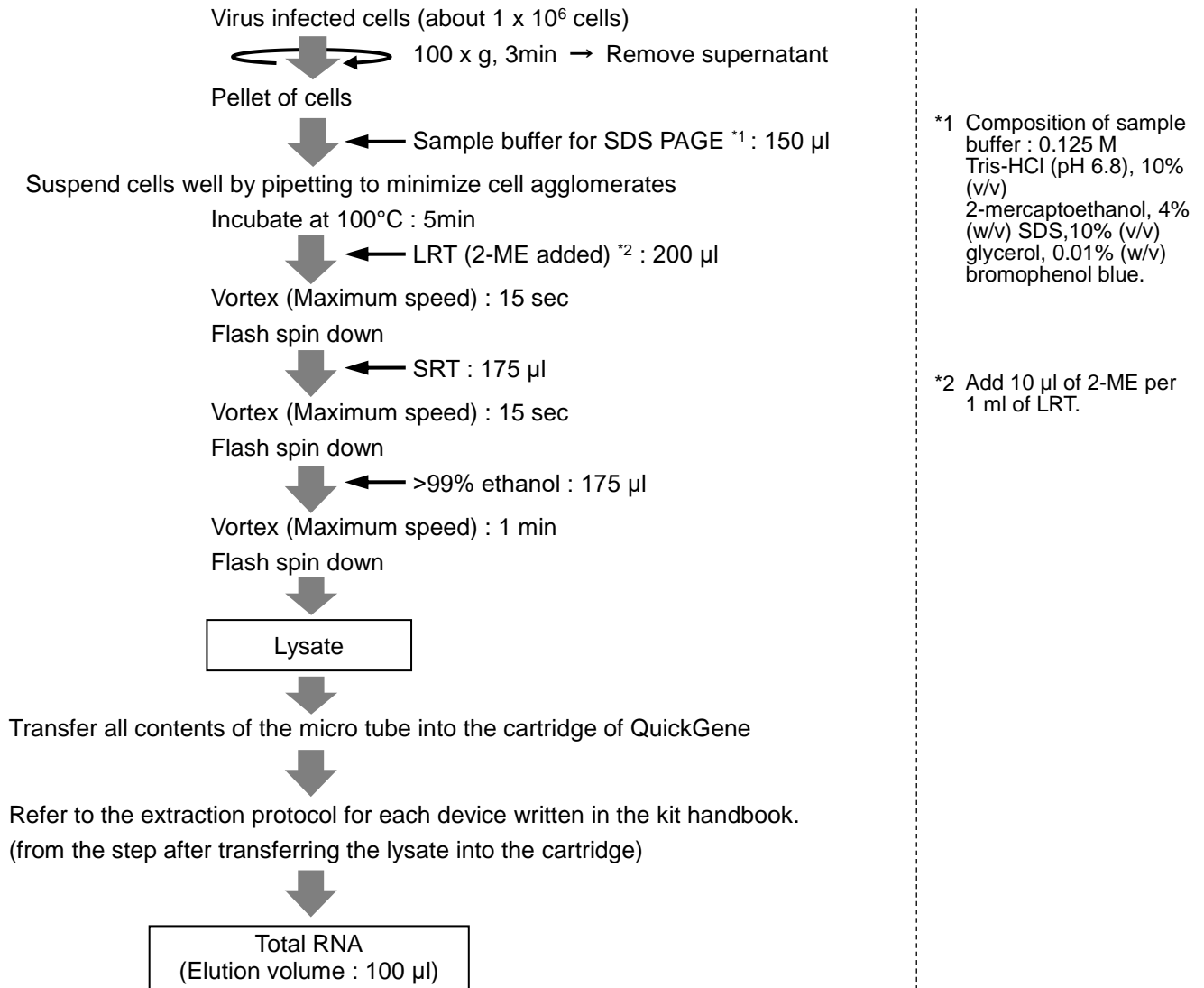
Common protocol is usable for the following

Measles Virus, Influenza Virus

RH-8

Total RNA Extraction from SARS Coronavirus (SARS-CoV) infected Cells

Protocol



Results

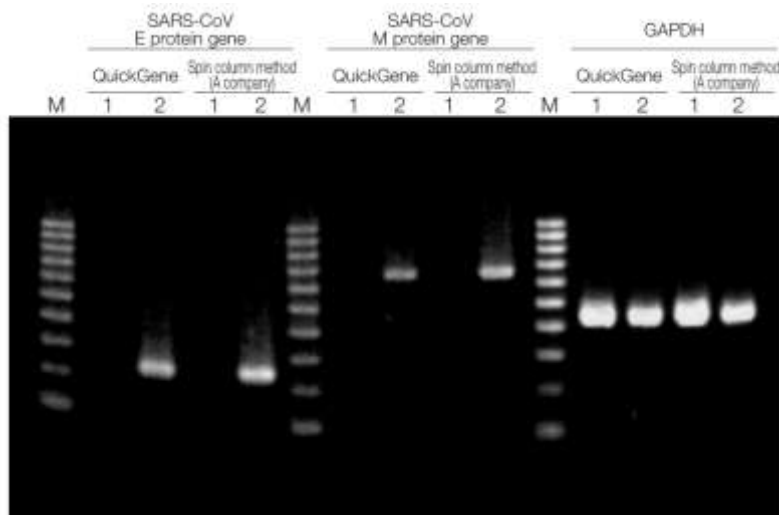
The yield of viral RNA / Protein contamination : A260/280

| Protocol | Yield (µg) | A260/280 |
|-----------------------------------|------------|----------|
| QuickGene | 9.4 | 1.93 |
| | 7.1 | 1.90 |
| Spin column method (A Company) | 7.6 | 1.80 |
| | 7.8 | 1.88 |

Other

▪ RT-PCR

RT-PCR was performed with primer characteristic to E protein gene and M protein gene of SARS-CoV, *GAPDH* gene for total RNA extracted from SARS-CoV infected cells using QuickGene system and Spin column method (A company).



Electrophoresis condition :
2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
1 : No.1 Noninfected Caco-2 cell
2 : No.2 SARS-CoV infected Caco-2 cell

RT-PCR products of E protein gene and M protein gene of SARS-CoV were detected for each total RNA of SARSCoV infected cell.

Common protocol is usable for the following

No data

RH-9

Viral RNA Extraction from Simian Immunodeficiency Virus (SIV) Infected Cells

Protocol

Place cells into 1.5 ml micro tube and pelletize
(~ 1 x 10⁶ cells in 1.5 ml micro tube)

↓ ← LRC (2-ME added) *1 : 350 µl

Vortex (Maximum speed) : 1 min *2

Flash spin down

↓ ← 70% ethanol : 350 µl

Vortex (Maximum speed) : 1 min *2

Flash spin down

Lysate *2

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Total RNA
(Elution volume : 100 µl)

*1 Add 10 µl of 2-ME per 1 ml of LRC.

*2 Mix completely by vortexing at the maximum speed. If the mixing is not enough by vortexing, use tapping, pipetting or inverting.

Results

The yield of viral RNA (µg)

| | Experiment 1 | | | Experiment 2 | | | |
|---------------|--------------|-------------|-------------|--------------|-------------|-----|-----|
| | mock | SIV clone 1 | SIV clone 2 | mock | SIV clone 2 | | |
| QuickGene-810 | 5.6 | 3.8 | 7.0 | 8.0 | 3.6 | 6.0 | 9.5 |
| Spin column | - | - | - | 7.1 | 0.8 | 4.5 | 4.7 |

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).

Protein contamination : A260/280

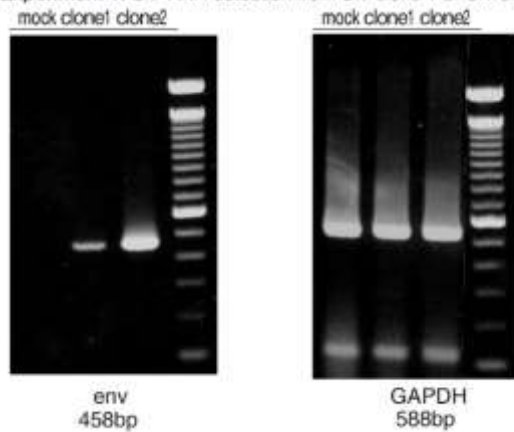
| Virus | Experiment 1 | | | Experiment 2 | | | |
|---------------|--------------|-------------|-------------|--------------|------|-------------|------|
| | mock | SIV clone 1 | SIV clone 2 | mock | | SIV clone 2 | |
| QuickGene-810 | 1.86 | 1.82 | 1.84 | 1.90 | 1.86 | 1.77 | 1.91 |
| Spin column | - | - | - | 1.92 | 1.66 | 1.82 | 1.88 |

Other

RT-PCR

AGE of RT-PCR with SIV-RNA isolated from SIV clone 1 or SIV clone 2 infected cells.

Experiment 1: SIV-RNA detection from SIV clone 1 or SIV clone 2 infected cells.

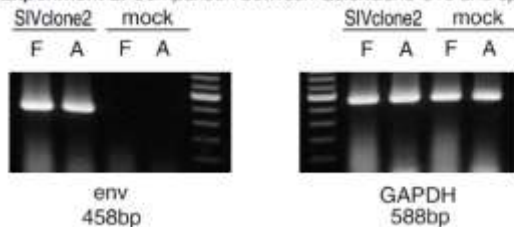


RT-PCR was performed on 1 µg of isolated total RNA.

RT-PCR amplification was performed successfully using total RNA.

As SIV clone 2 has higher infectiousness than SIV clone 1, larger amount of SIV-RNA can be isolated from SIV clone 2 infected cells.

Experiment 2: Comparison between QuickGene-810 and spin column



F : QuickGene-810

A : Spin column

Isolated S2V-RNA was used for RT-PCR template to amplify env and GAPDH gene.

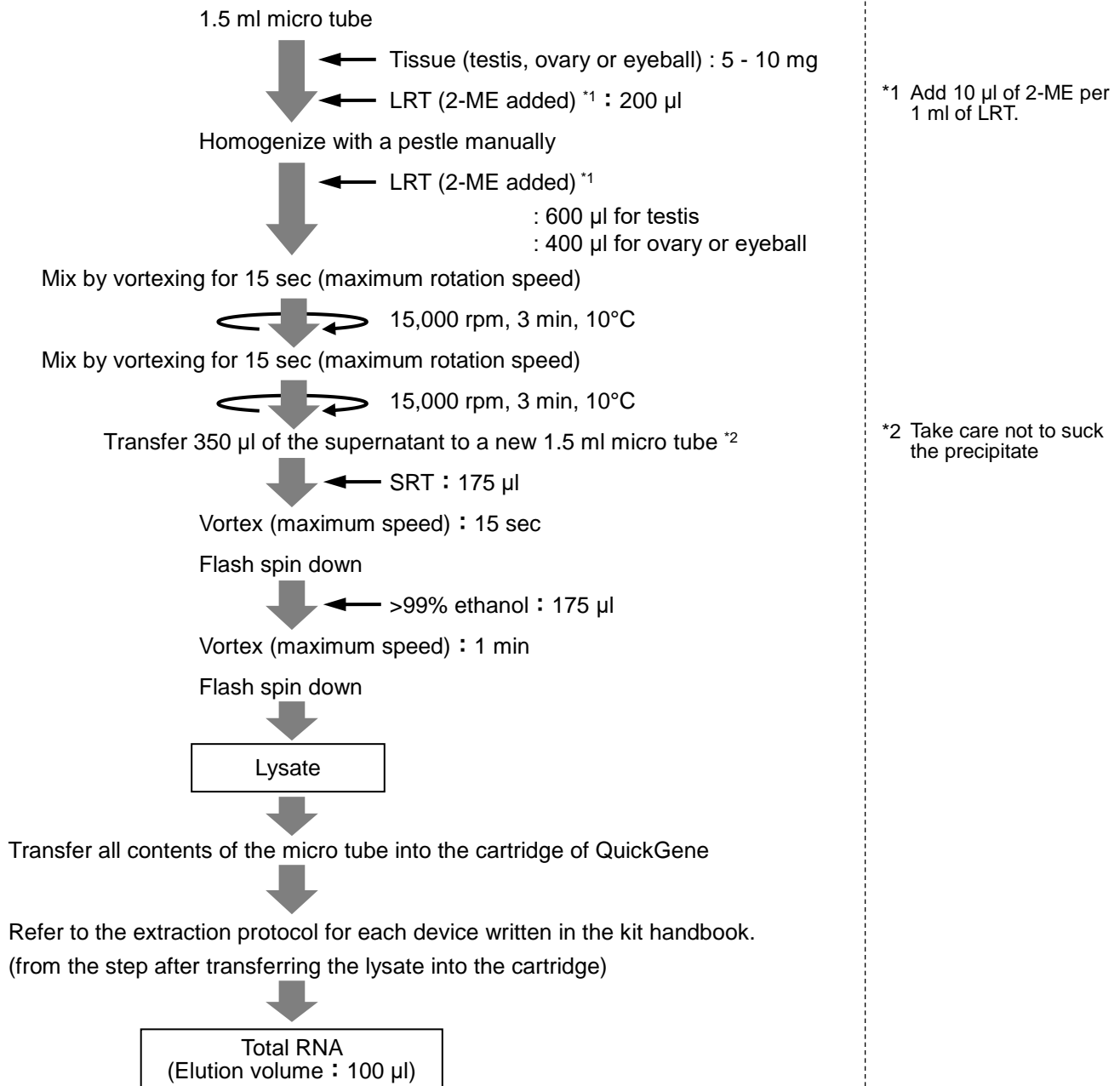
Common protocol is usable for the following

No Data

RH-10

VNN (Viral Nervous Necrosis) RNA Extraction from Tilefish

Protocol



Results

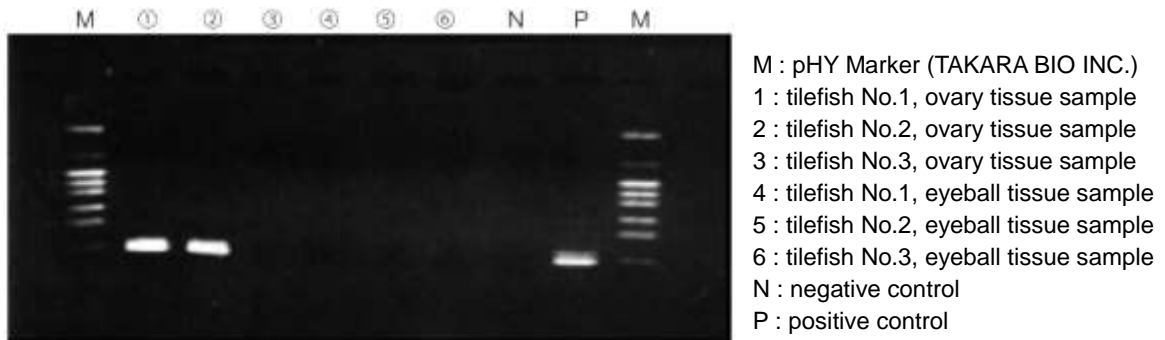
Other

Inspection

RT-PCR : Amplification was performed on isolated RNA, targeting T4 region of RGNNV coat protein gene.

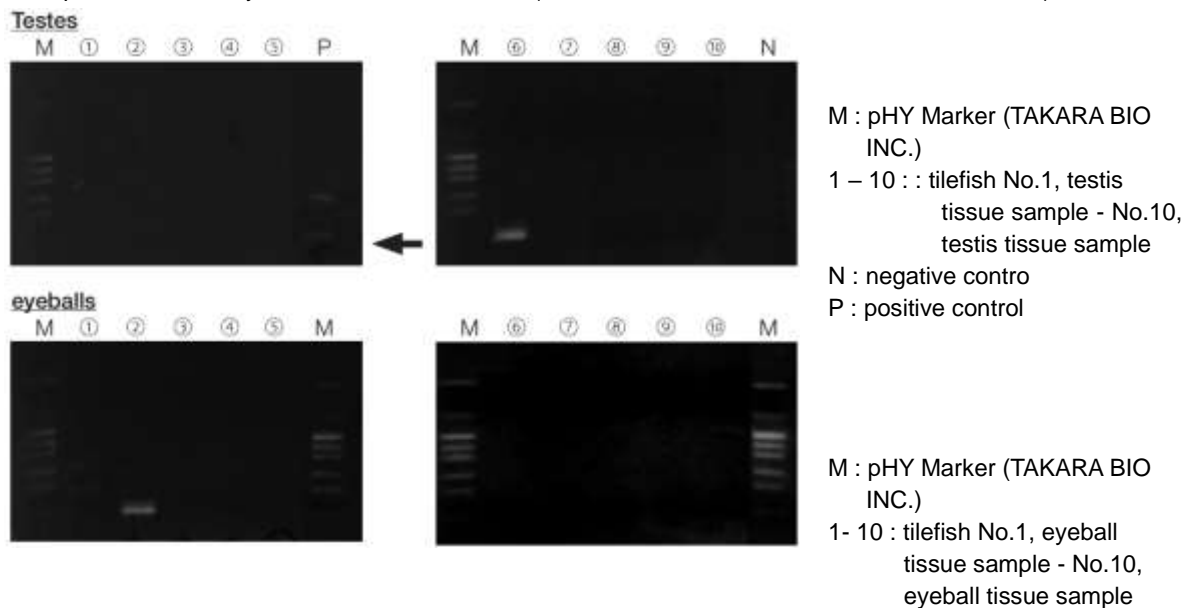
Nested PCR : Amplification was performed using the primer specific to RG type among 4 genotypes of betanodavirus.

Sample : ovaries and eyeballs of 3 native tilefish ♀ (each tissue was taken from the same individual)



Result : Amplification products similar to those of positive control were confirmed for ovaries of No.1, 2.

Sample : testes and eyeballs of 10 native tilefish (each tissue was taken from the same individual)



Result : Amplification products were confirmed for testes of No.6 and eyeballs of No.2.

Common protocol is usable for the following

No Data