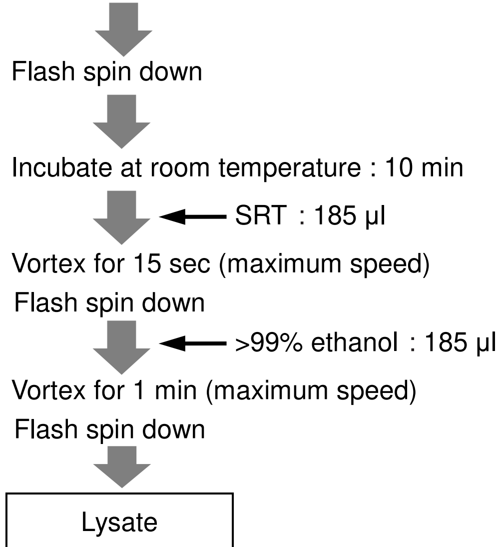
18. Total RNA Extraction from Virus

RH-1

Hepatitis C Virus (HCV) RNA Extraction from Serum

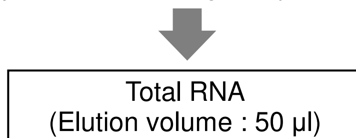
Protocol

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.

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).

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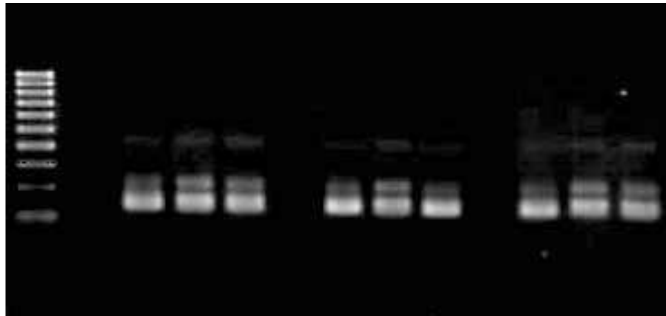
Results

Other

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

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

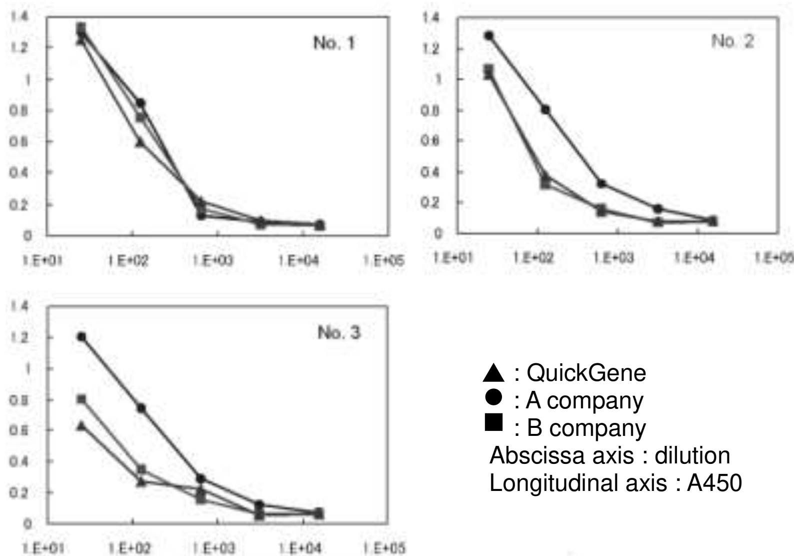


M	: marker (100 bp ladder)
a, e, i	: HCV negative normal human
b, f, j	: HCV positive patient No.1
c, g, k	: HCV positive patient No.2
d, h, l	: HCV positive patient No.3
a ~ d	: QuickGene
e ~ h	: A company
i ~ l	: B company

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).

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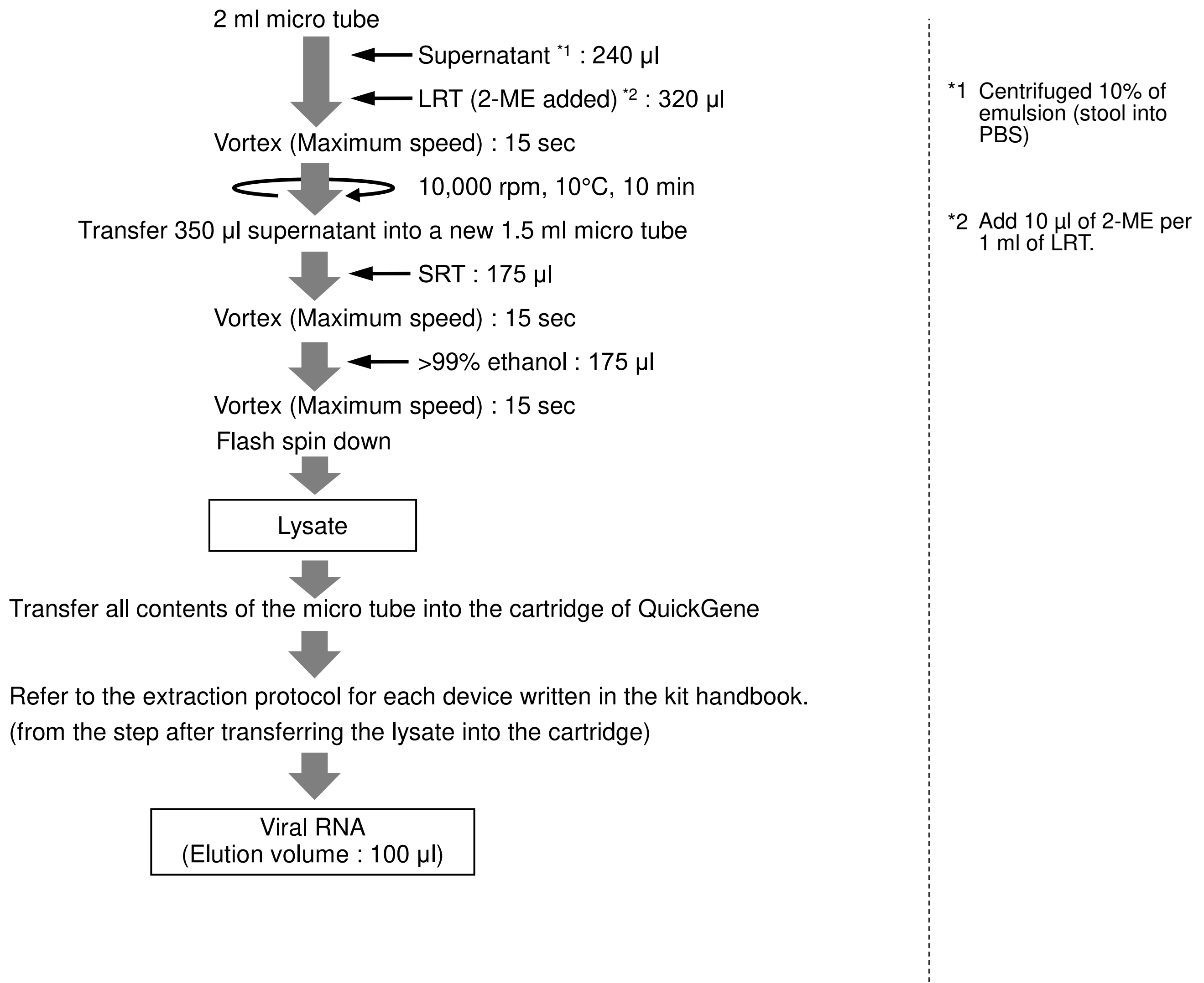
RH-2

Norovirus RNA Extraction from Stool

Protocol A (PCR Method)



*1 Centrifuged 10% of emulsion (stool into PBS)

*2 Add 10 µl of 2-ME per 1 ml of LRT.

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

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).

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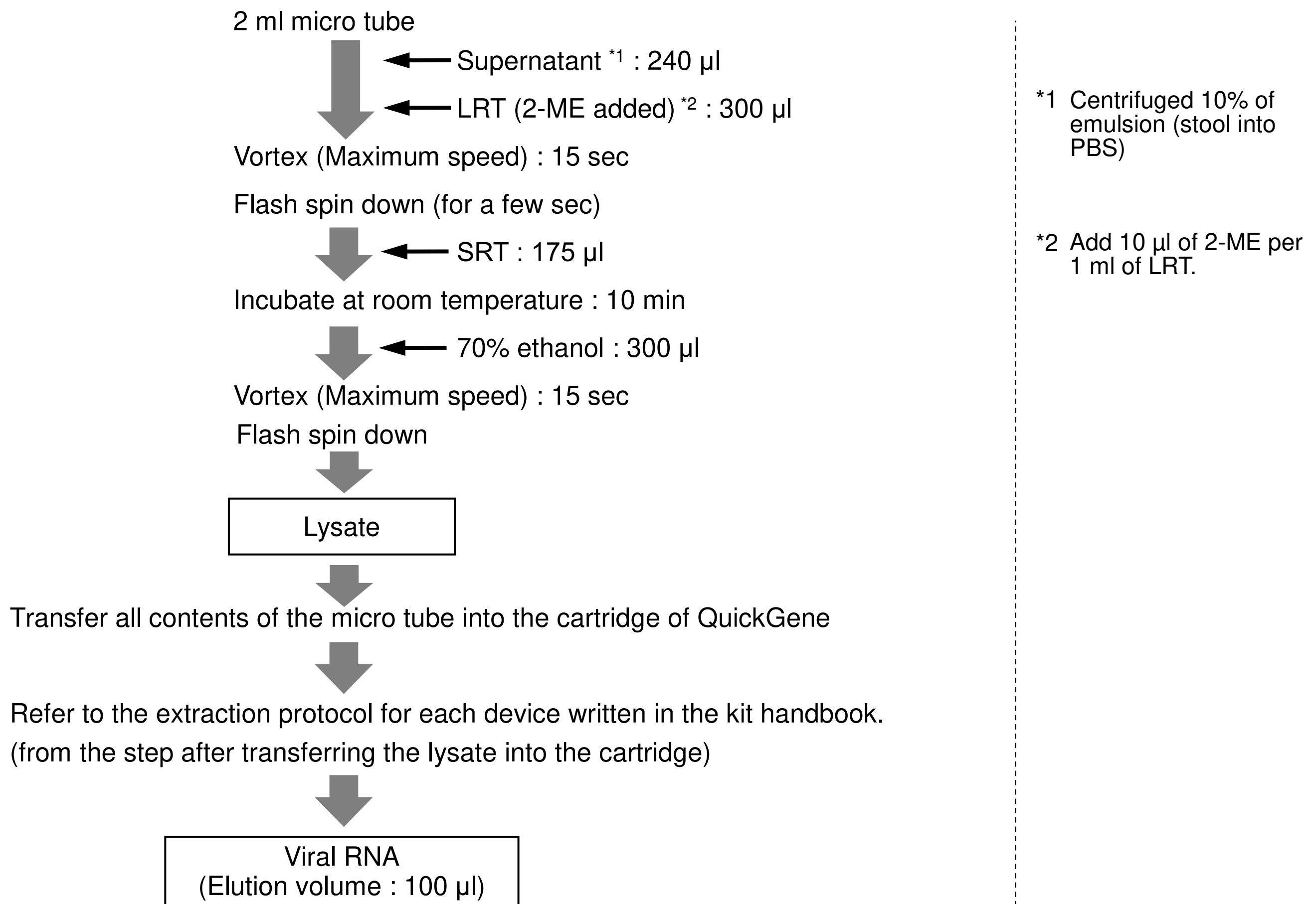
Protocol B (TRC Method)



*1 Centrifuged 10% of emulsion (stool into PBS)

*2 Add 10 µl of 2-ME per 1 ml of LRT.

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

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).

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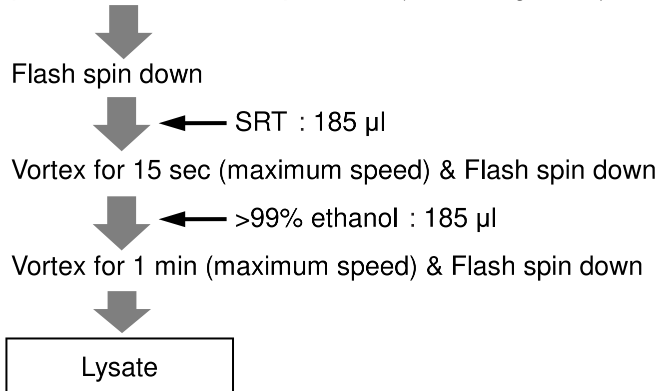
RH-3

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 preparted 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).

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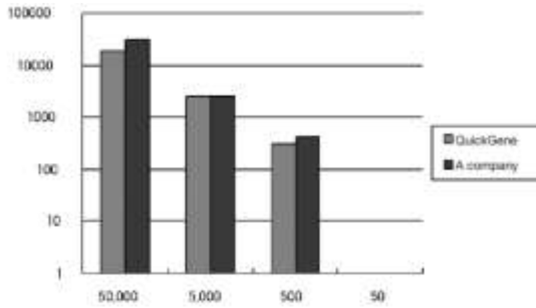


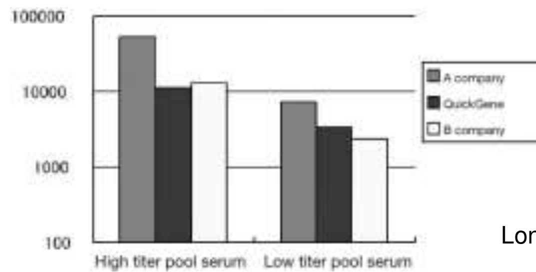
Abscissa axis : spiked virus particle amount
Longitudinal axis : calculated HIV RNA amount

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.

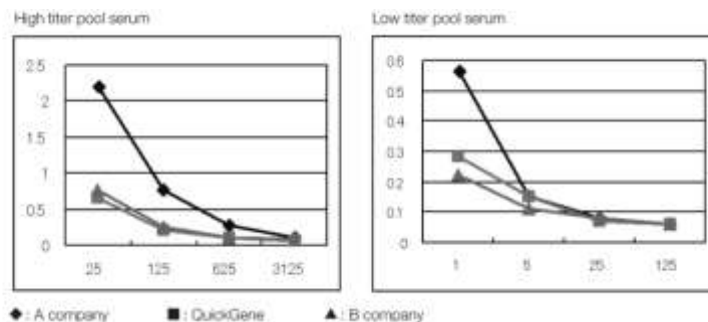
▪ Refinement of HIV RNA from human serum which spiked HIV virus particle

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	3349.9
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).

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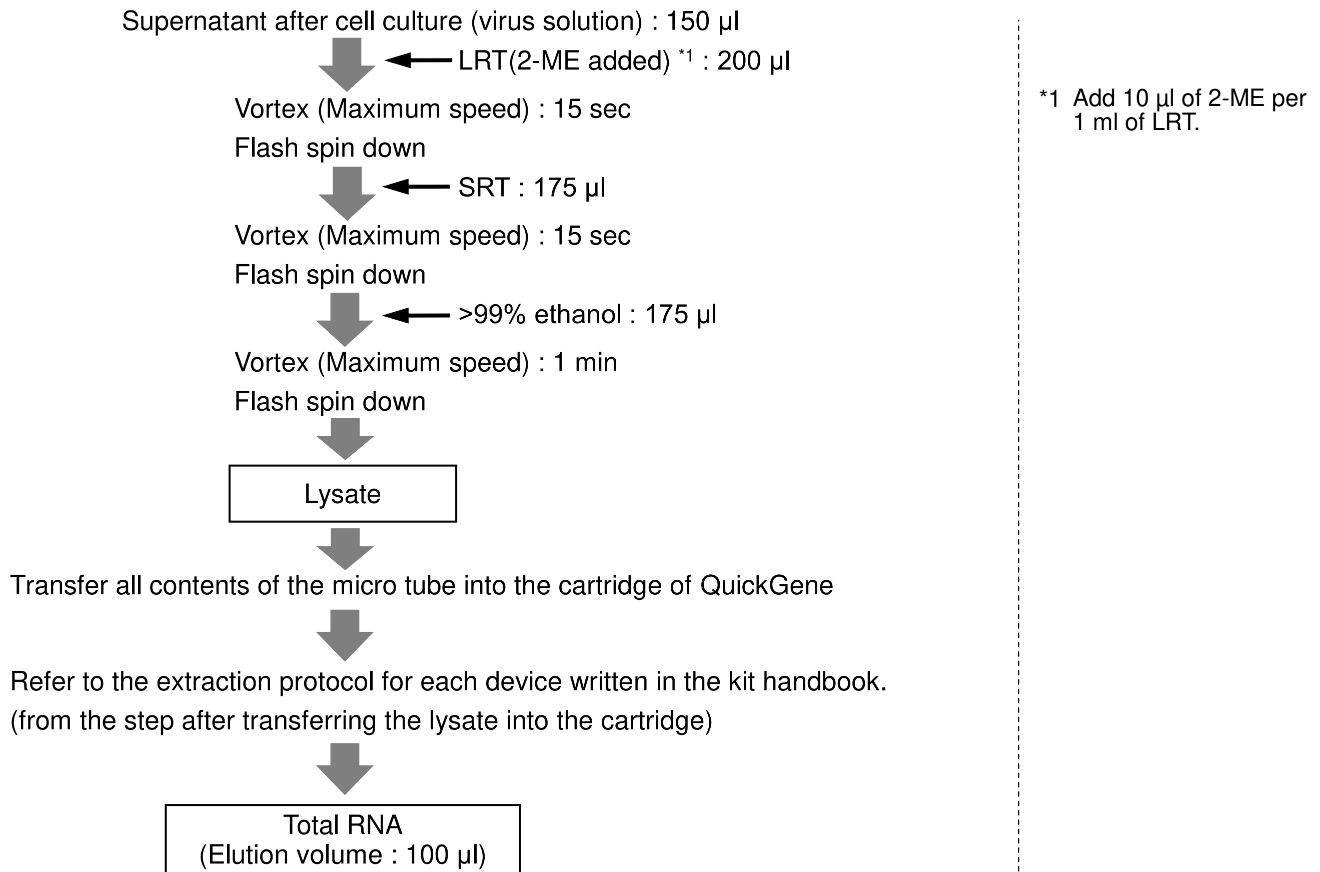
Total RNA Extraction from Influenza Virus Solution

Protocol



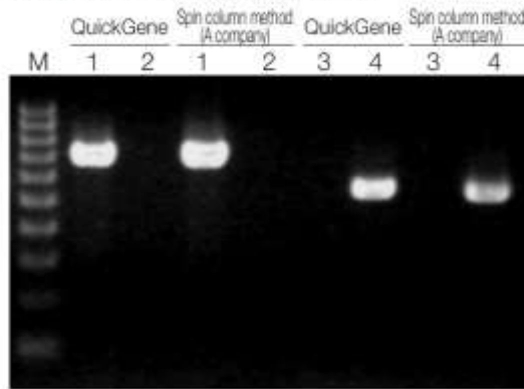
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

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).

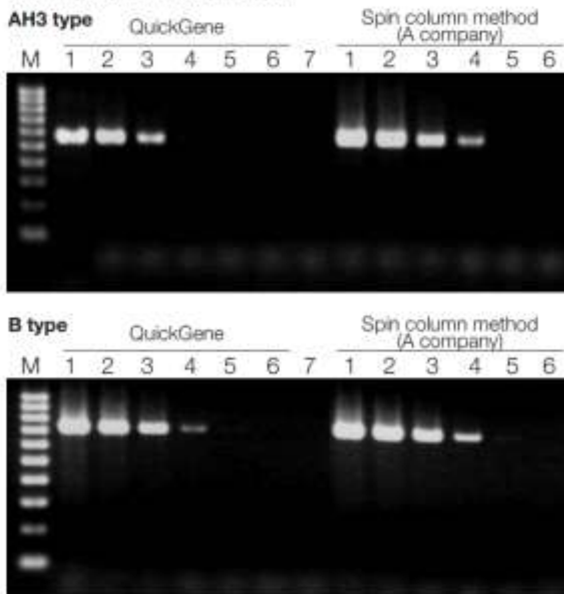


Electrophoresis condition : 2.0% agarose/1 x TAE

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.



Electrophoresis condition : 2.0% agarose/1 x TAE

M: 100 bp DNA Ladder
1 : Influenza virus, 10^6 pfu/ml
2 : Influenza virus, 10^5 pfu/ml
3 : Influenza virus, 10^4 pfu/ml
4 : Influenza virus, 10^3 pfu/ml
5 : Influenza virus, 10^2 pfu/ml
6 : Influenza virus, 10^1 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

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).

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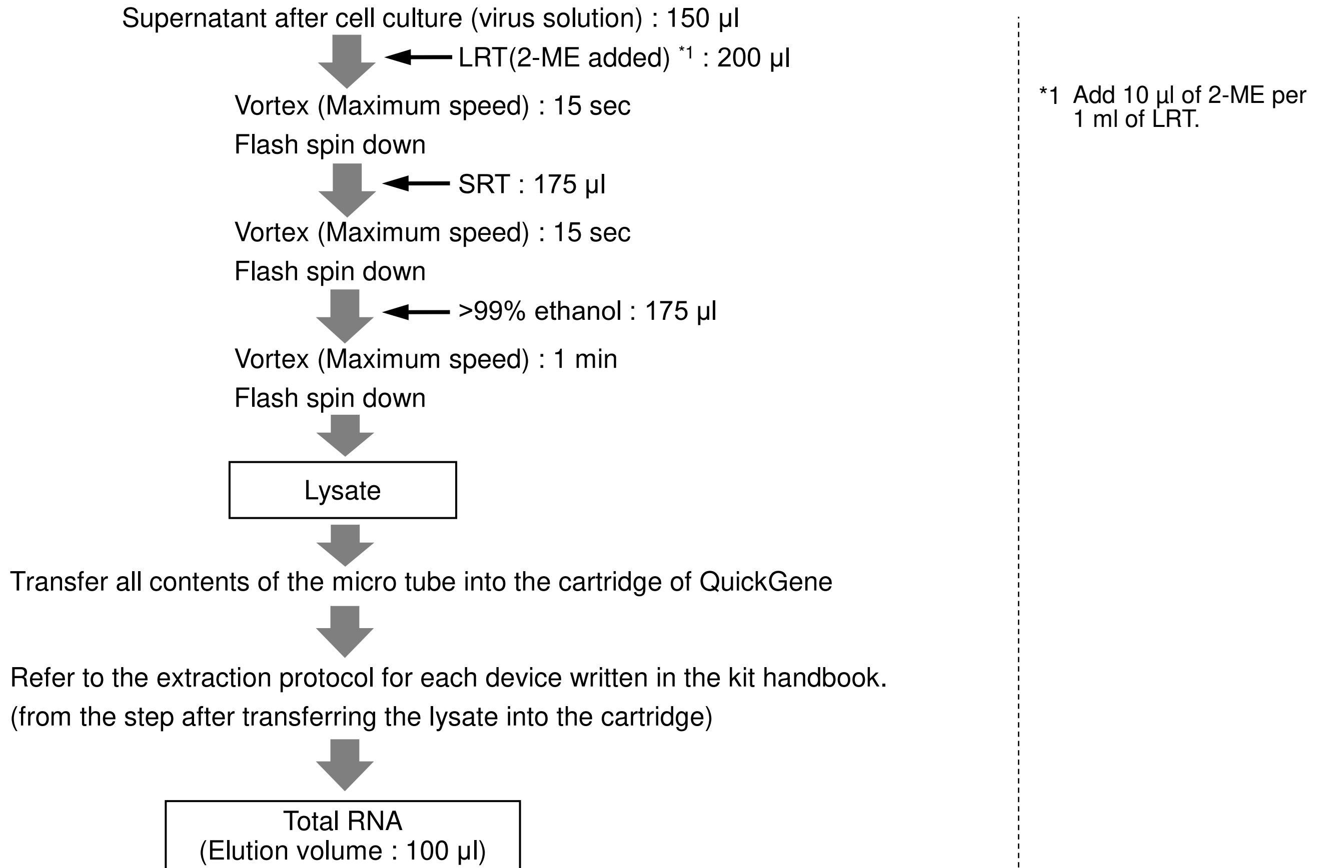
RH-5

Total RNA Extraction from Measles Virus Solution

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

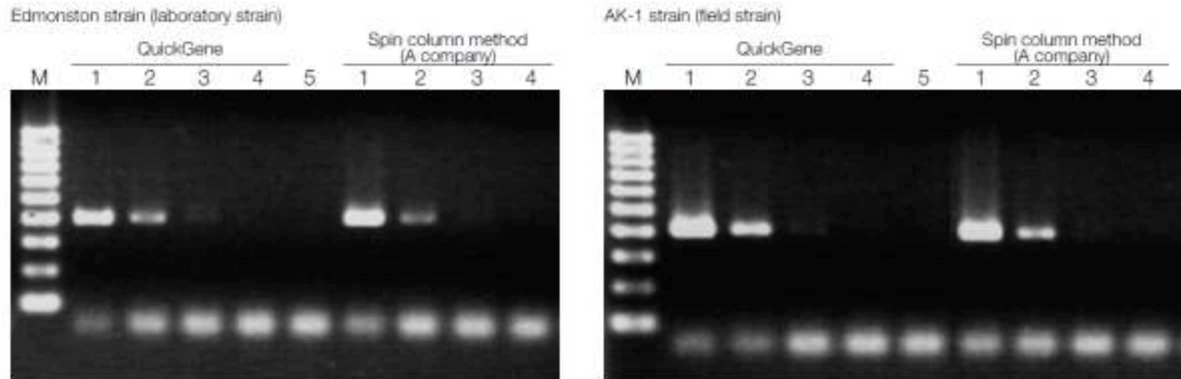
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

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.

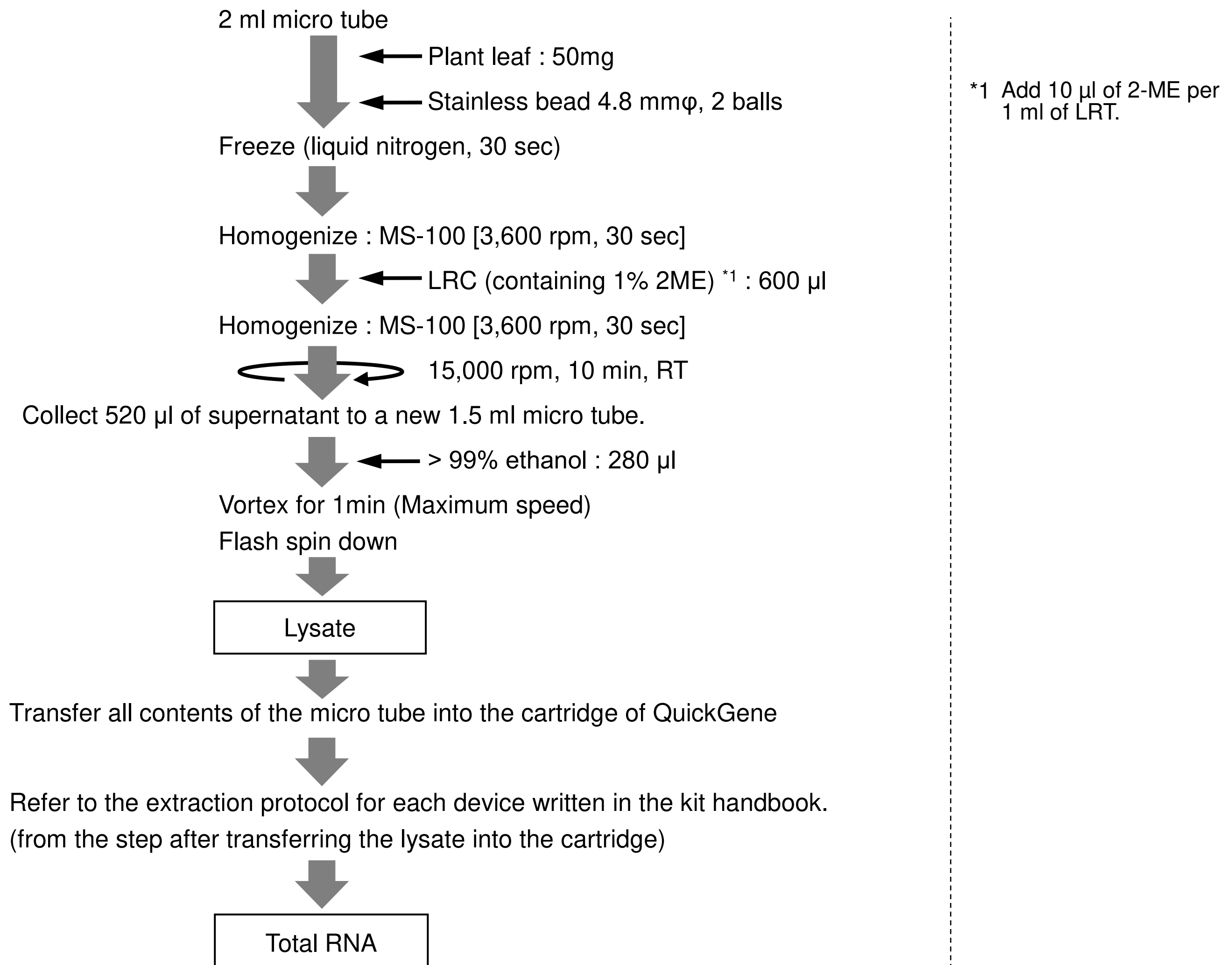
Common protocol is usable for the following

Influenza Virus, Respiratory Syncytial (RS) Virus

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).

Total RNA Extraction from Plant virus

Protocol



Results

No Data

Common protocol is usable for the following

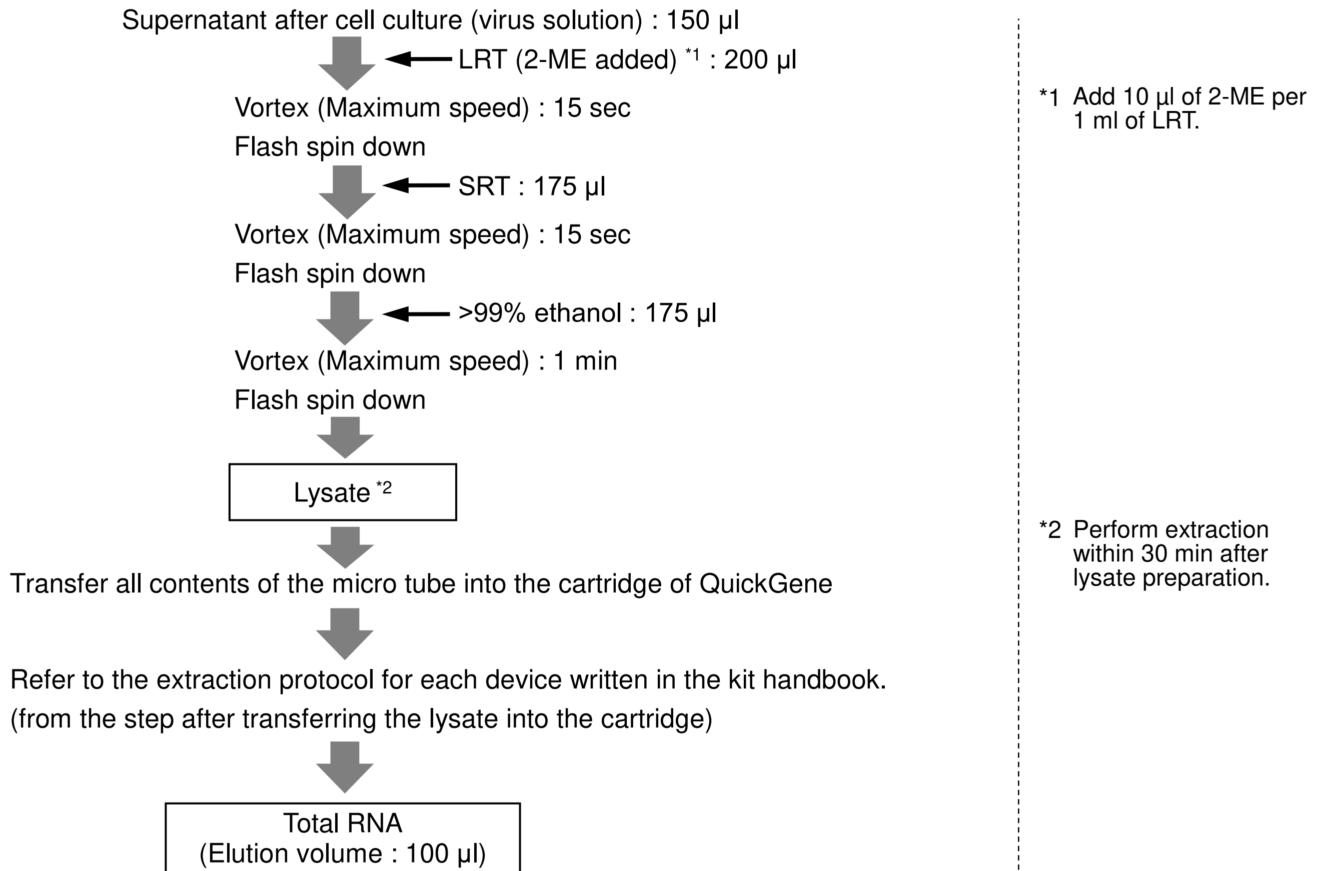
No Data

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-7

Total RNA Extraction from Respiratory Syncytial (RS) Virus Solution

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

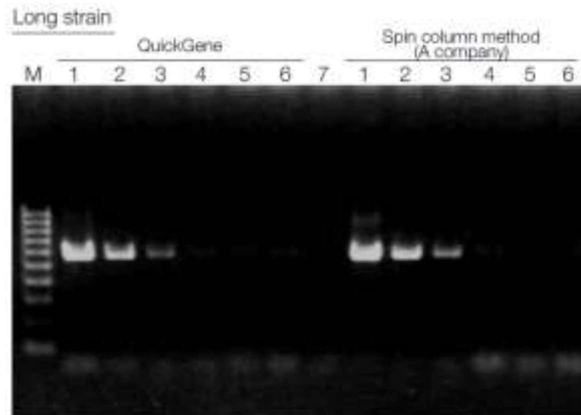
*2 Perform extraction within 30 min after lysate preparation.

Results

Other

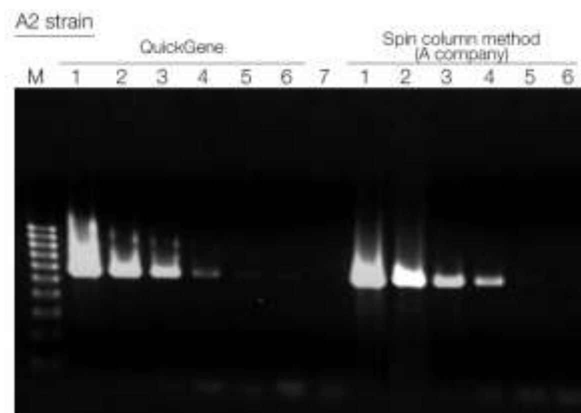
▪ RT-PCR

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, 10^5 pfu/ml
2 : RC virus, 10^4 pfu/ml
3 : RC virus, 10^3 pfu/ml
4 : RC virus, 10^2 pfu/ml
5 : RC virus, 10^1 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.

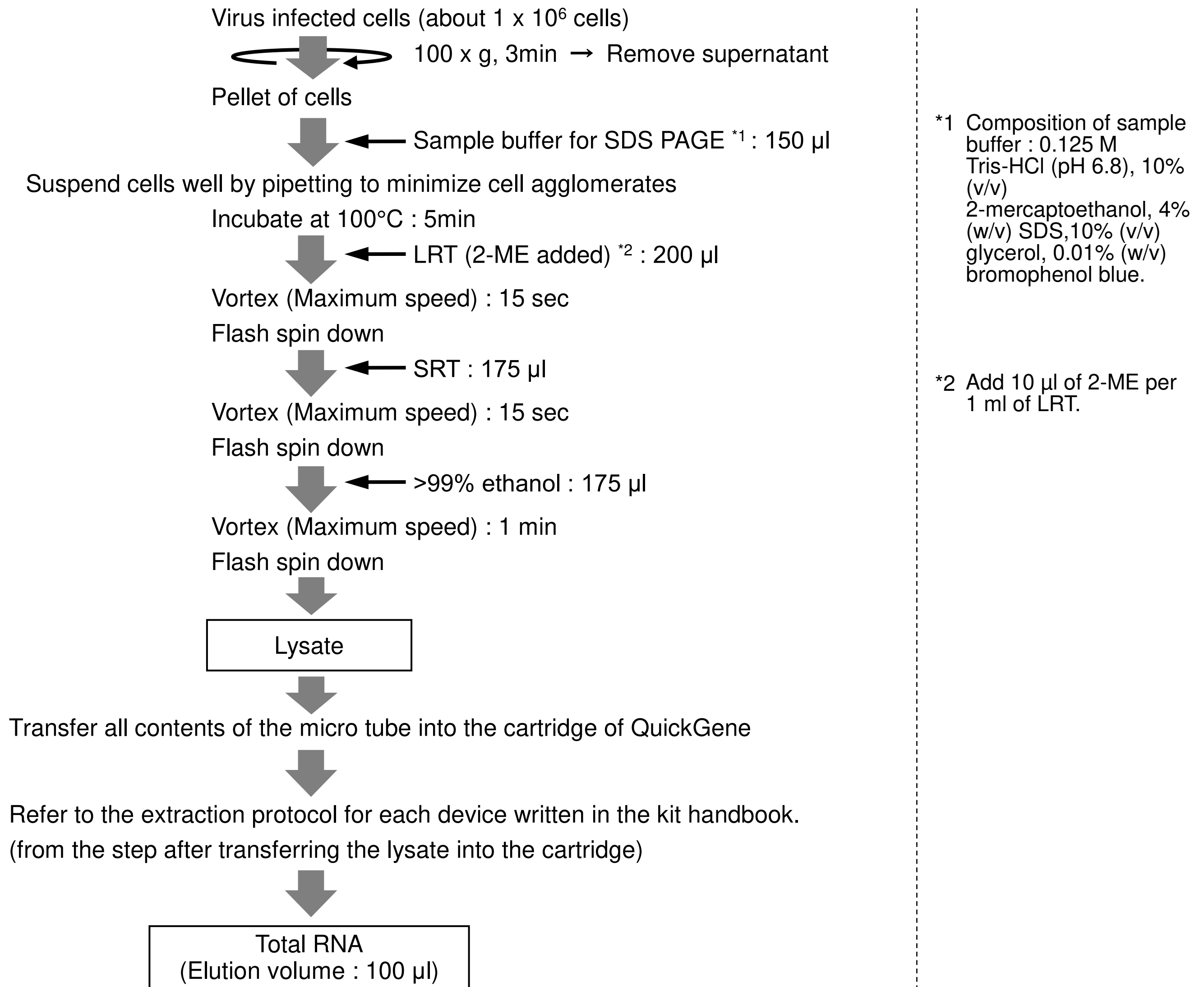
Common protocol is usable for the following

Measles Virus, Influenza Virus

RH-8

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

Protocol



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).

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Results

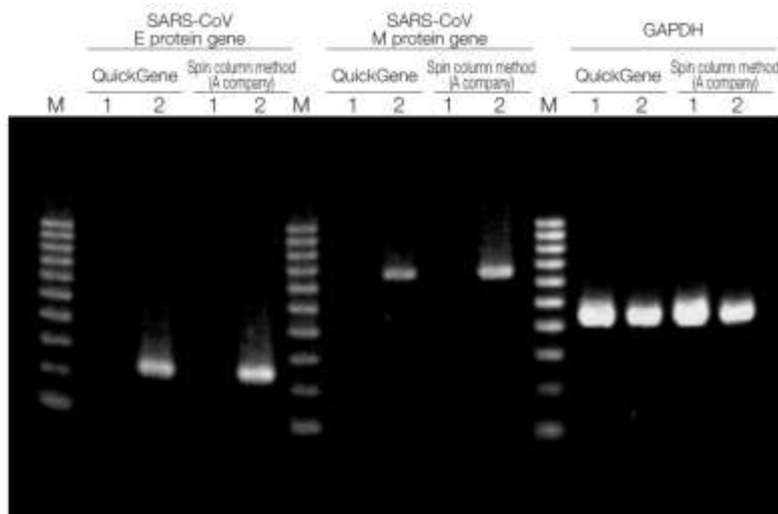
The yield of viral RNA / Protein contamination：A260/280

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

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).

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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 10^6 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			
Virus	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).

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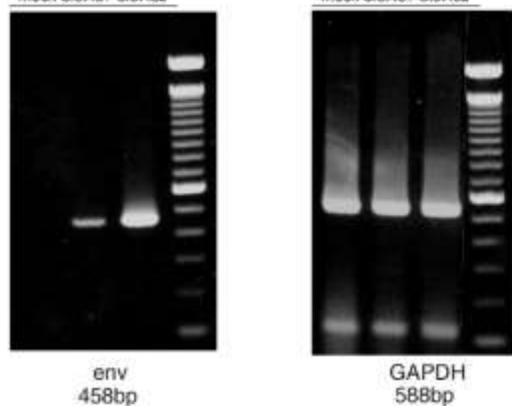
Protein contamination : A260/280

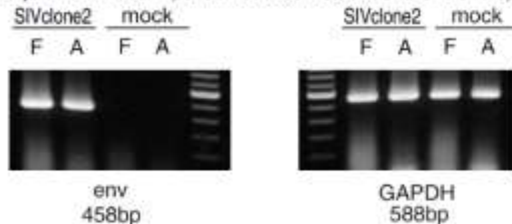
	Experiment 1			Experiment 2			
Virus	mock	SIV clone 1	SIV clone 2	mock		SIV clone 2	
QuickGene-810	1.86	1.82	1.84	1.90	1.86	1.77	1.91
Spin column	-	-	-	1.92	1.66	1.82	1.88

Other

- RT-PCR

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





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

RT-PCR amplification was performed succesfully using total RNA.

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

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

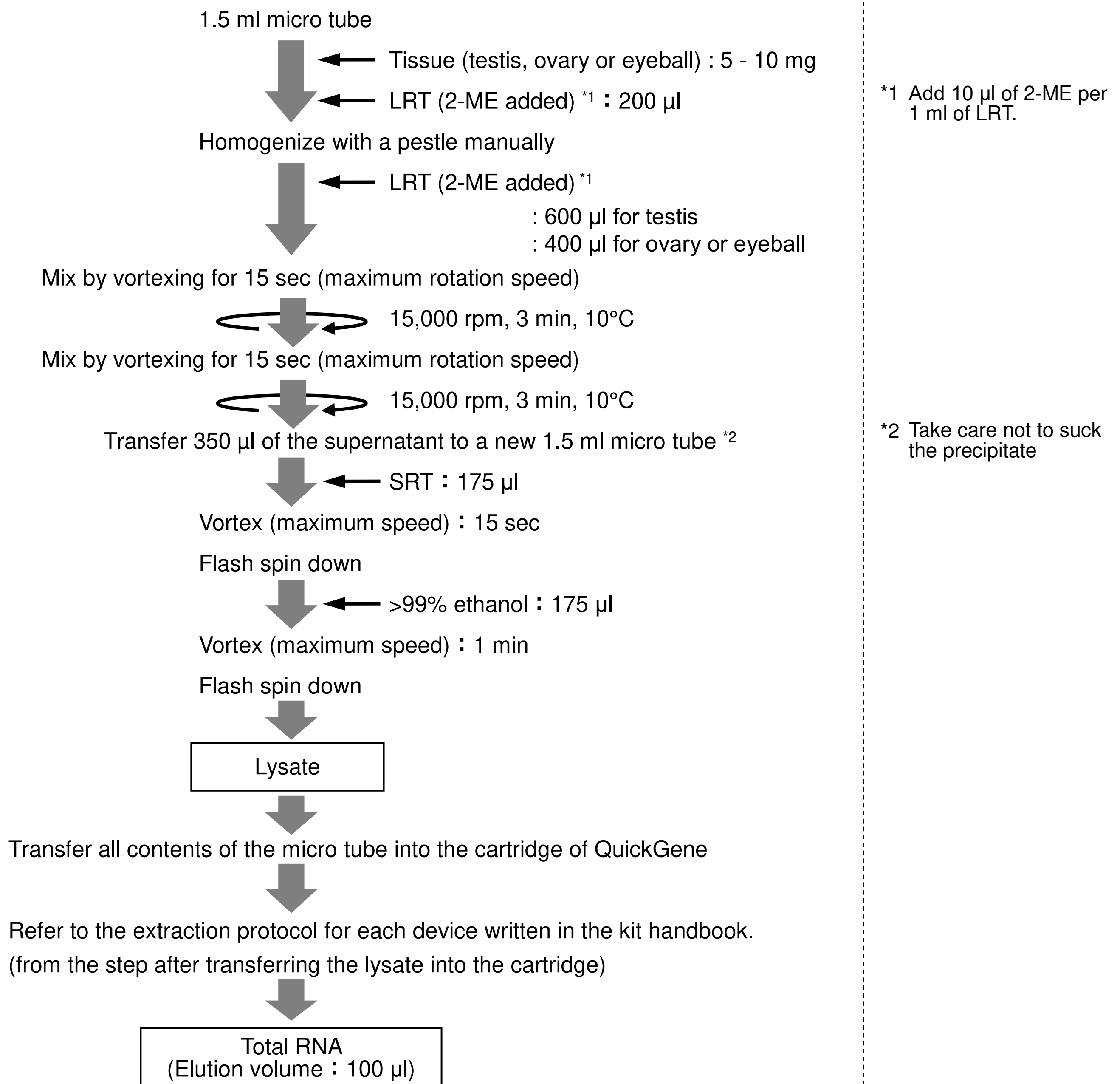
No Data

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-10

VNN (Viral Nervous Necrosis) RNA Extraction from Tilefish

Protocol



*1 Add 10 µl of 2-ME per 1 ml of LRT.

*2 Take care not to suck the precipitate

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).

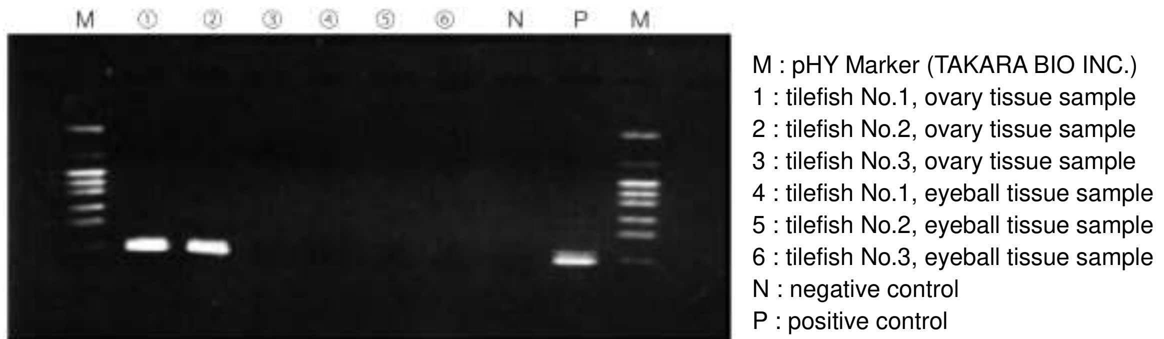
Results

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.

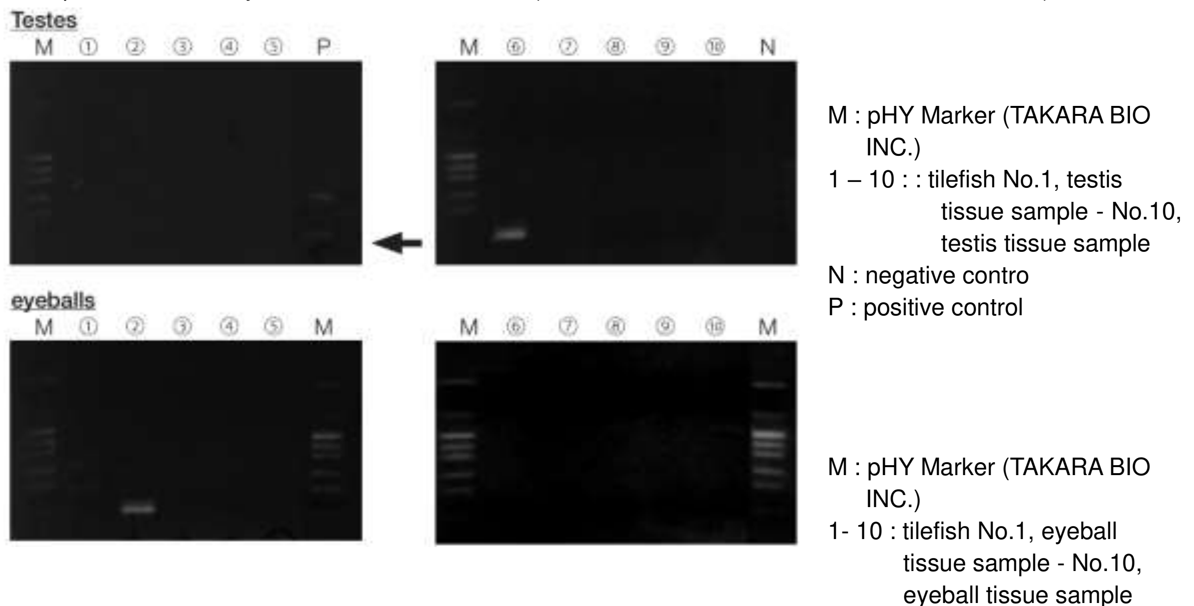
Sample : ovaries and eyeballs of 3 native tilefish ♀ (each tissue was taken from the same individual)



M : pHY Marker (TAKARA BIO INC.)
1 : tilefish No.1, ovary tissue sample
2 : tilefish No.2, ovary tissue sample
3 : tilefish No.3, ovary tissue sample
4 : tilefish No.1, eyeball tissue sample
5 : tilefish No.2, eyeball tissue sample
6 : tilefish No.3, eyeball tissue sample
N : negative control
P : positive control

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)



M : pHY Marker (TAKARA BIO INC.)
1 – 10 : : tilefish No.1, testis tissue sample - No.10, testis tissue sample
N : negative contro
P : positive control

M : pHY Marker (TAKARA BIO INC.)
1- 10 : tilefish No.1, eyeball tissue sample - No.10, eyeball tissue sample

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

Common protocol is usable for the following

No Data

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).

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