Purification of viral RNA from fluid biological samples

ISOSPIN Viral RNA

INSTRUCTION MANUAL Version 1

Code No. 310-08931

NIPPON GENE CO., LTD.

I Introduction

ISOSPIN Viral RNA kit is designed for the rapid preparation of viral RNA from fluid biological samples, such as nasopharyngeal and oropharyngeal swab washes, serum, saliva, and sputum.

With the ISOSPIN Viral RNA method, RNA viruses are lysed quickly by NIRV Extraction Buffer and Proteinase K. Ethanol is added to the lysate and the lysate and ethanol create appropriate conditions for binding of RNA to the silica-based membrane of the Spin Column. Impurities are removed by washing with two kinds of Wash Buffers. The RNA is eluted in RNase-free water.

The Spin Column of the ISOSPIN Viral RNA kit enables a high loading capacity. ISOSPIN Viral RNA kit allows purification of pure RNA in 30 minutes without phenol/chloroform extraction or alcohol precipitation.

Advantages

- Designed to purify viral RNA in 30 min
- High loading capacity of Spin Column and good operability
- Ideal for sensitive applications such as RT-qPCR

Works for a wide variety of viruses, including:

- Coronavirus (SARS-CoV-2)
- Poliovirus
- Hepatitis E virus (HEV)
- Severe febrile thrombocytopenia syndrome virus (SFTSV)

II Kit Components

ISOSPIN Viral RNA (Code No. 310-08931)

Item	Amount (50 preps)	Storage Temperature
Proteinase K	1 ml	–20°C
NIRV Extraction Buffer	18 ml	Room temperature
NIRV Wash1 Buffer *	30 ml	Room temperature
NIRV Wash2 Buffer *	30 ml	Room temperature
ddWater	3 x 1 ml	Room temperature
Spin Column †	50	Room temperature
Collection Tube	2 x 50	Room temperature

* Contains ethanol. Always keep buffer bottles tightly closed.

† Contains columns, plus Collection Tube.

Shipping and Storage

All components of the ISOSPIN Viral RNA kit are shipped at room temperature. Upon receipt, store Proteinase K at –20°C.

Note

If you want to premix NIRV Extraction Buffer with Proteinase K, you can store the premixed NIRV Extraction Buffer containing Proteinase K at –80°C for up to 5 days. Do not store the premixed NIRV Extraction Buffer containing Proteinase K at –20°C.

III Caution

- This product is intended for general laboratory use and research use only. Not intended for any animal or human therapeutic or diagnostic use.
- Please observe general laboratory precautions, and follow safety guidelines while using this kit.
- NIPPON GENE does not assume any responsibility for damages due to improper application of our products in other fields of application.
- Please see the Safety Data Sheets available on our website (www.nippongene.com).

IV Protocol

The ISOSPIN Viral RNA purification procedure is carried out using Spin Columns and standard microcentrifuge. All centrifugation steps are performed at 8,000 x g at room temperature.

Sample types include:

- Sputum (pretreated suputum)
- Nasopharyngeal swab (with transport medium for viruses)
- Oropharyngeal swab washes
- Saliva
- Serum
- Other cell-free body fluids
- Cell-culture supernatants

Equipment and reagents to be supplied by user

Reagents

- 96-100% ethanol

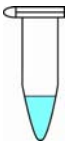
Consumables

- Pipette tips
- 1.5 ml microcentrifuge tubes

Equipment

- Manual pipettes
- Vortex mixer
- Benchtop Centrifuge
- Microcentrifuge
- Heat block set to 56°C (For sputum samples etc.)

Protocol: Purification of Viral RNA from 140 µl samples



1. Add 20 µl of Proteinase K into a 1.5 ml tube.
   Add 360 µl of NIRV Extraction Buffer to the 1.5 ml tube.
   Add 140 µl of the fluid sample to the 1.5 ml tube. Vortex for 15 seconds.

2. Incubate samples at room temperature for 10 minutes.
   (For sputum samples, incubate at 56°C for 10 minutes)

3. Spin down briefly to collect any drops at the bottom of the tube.

4. Add 400 µl of ethanol. Vortex for 15 seconds. Spin down briefly.

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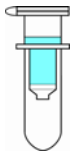

5. Carefully apply the whole tube contents (up to 920 µl of the lysate from step 4) to a Spin Column, cap it and place it in a microcentrifuge.

6. Centrifuge at 8,000 x g for 15 seconds at room temperature.

7. Discard the flow-through with Collection Tube. Place the column into a new Collection Tube.

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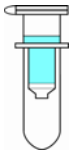

8. Add 500 µl of NIRV Wash1 Buffer to the column.

9. Centrifuge at 8,000 x g for 15 seconds at room temperature.

10. Discard the flow-through with Collection Tube. Place the column into a new Collection Tube.

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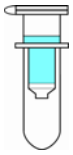

11. Add 500 µl of NIRV Wash2 Buffer to the column.

12. Centrifuge at 8,000 x g for 2 minutes at room temperature.

13. Discard the flow-through with Collection Tube.

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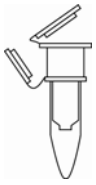


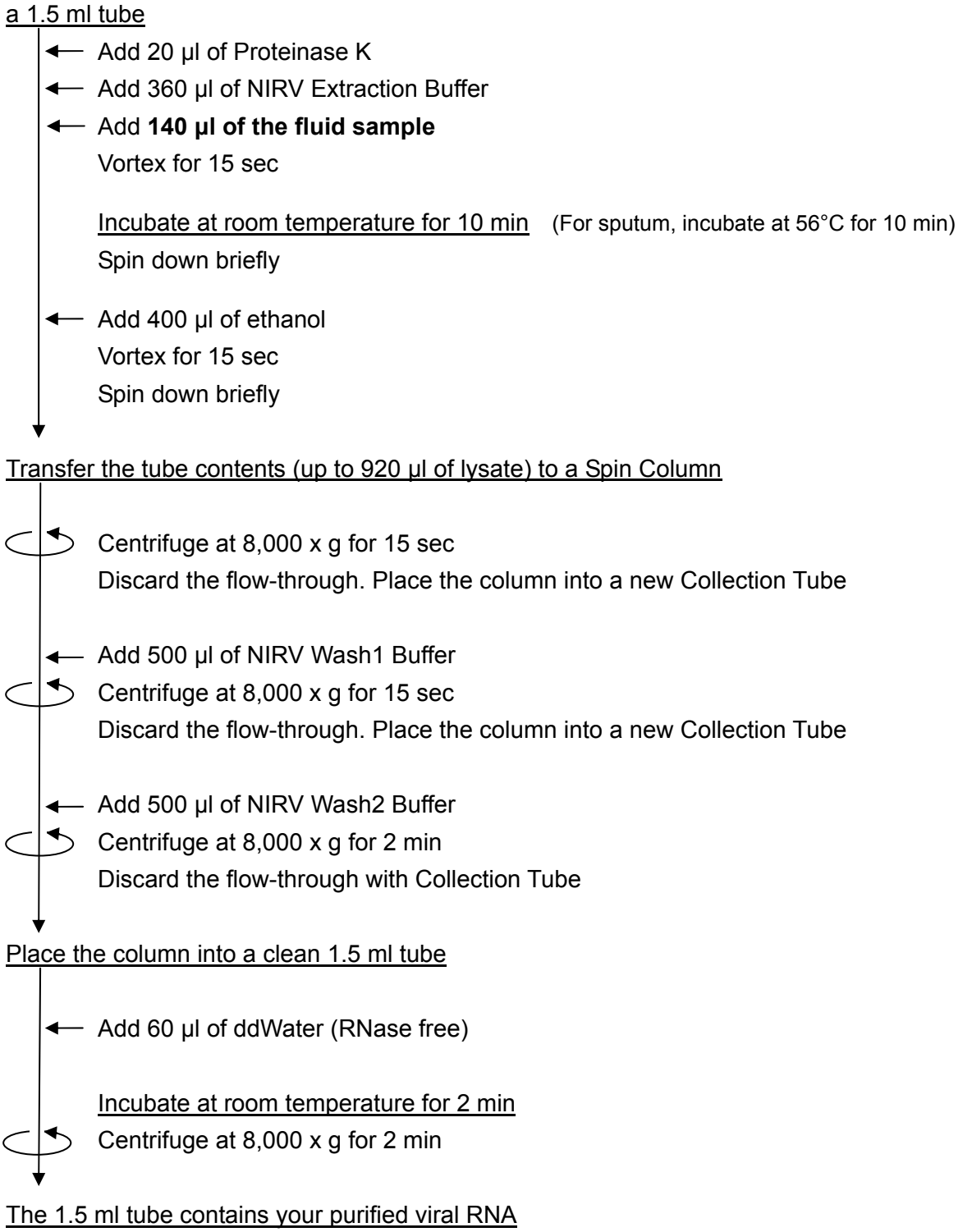
14. Place the column into a clean 1.5 ml tube.

15. Add 60 µl of ddWater (RNase free) to the center of the membrane. Incubate at room temperature for 2 minutes.

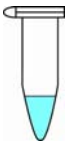
16. Centrifuge at 8,000 x g for 2 minutes at room temperature.

17. The 1.5 ml tube contains your purified viral RNA.

The flow chart describes the steps for purification of viral RNA from 140 µl samples.



Optional Protocol: Purification of Viral RNA from 250 µl samples *(Scale-Up)*



1. Add 20 µl of Proteinase K into a 1.5 ml tube.
   Add **350 µl of NIRV Extraction Buffer** to the 1.5 ml tube.
   Add **250 µl of the fluid sample** to the 1.5 ml tube. Vortex for 15 seconds.
2. Incubate samples at room temperature for 10 minutes.
   (For sputum samples, incubate at 56°C for 10 minutes)
3. Spin down briefly to collect any drops at the bottom of the tube.
4. Add **350 µl of ethanol**. Vortex for 15 seconds. Spin down briefly.

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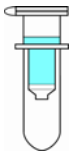

5. Carefully apply the whole tube contents (**up to 970 µl of the lysate** from step 4) to a Spin Column, cap it and place it in a microcentrifuge.
6. Centrifuge at 8,000 x g for 15 seconds at room temperature.
7. Discard the flow-through with Collection Tube. Place the column into a new Collection Tube.

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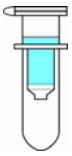

8. Add 500 µl of NIRV Wash1 Buffer to the column.
9. Centrifuge at 8,000 x g for 15 seconds at room temperature.
10. Discard the flow-through with Collection Tube. Place the column into a new Collection Tube.

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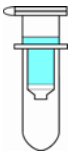

11. Add 500 µl of NIRV Wash2 Buffer to the column.
12. Centrifuge at 8,000 x g for 2 minutes at room temperature.
13. Discard the flow-through with Collection Tube.

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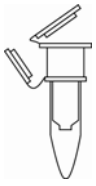
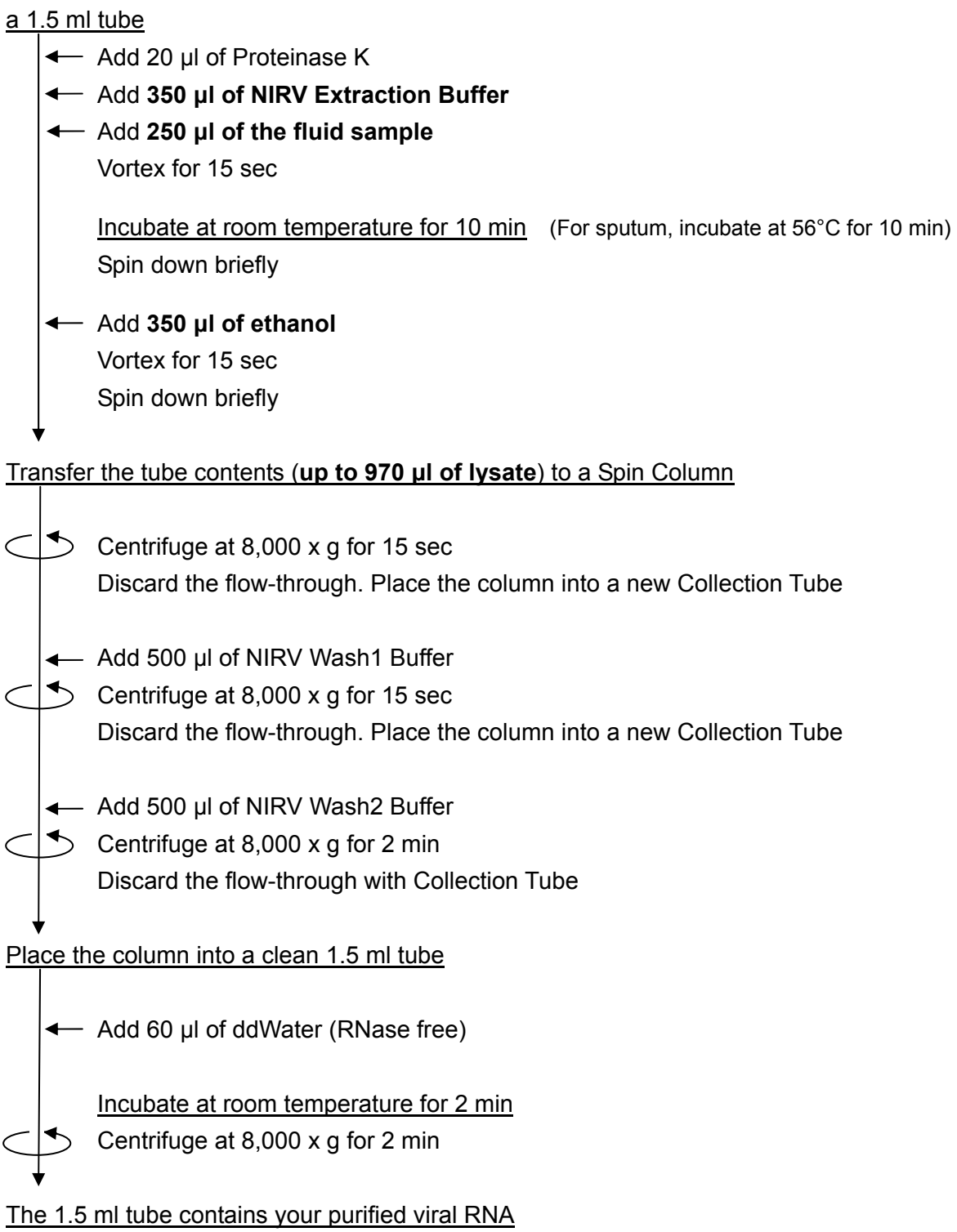


14. Place the column into a clean 1.5 ml tube.
15. Add 60 µl of ddWater (RNase free) to the center of the membrane. Incubate at room temperature for 2 minutes.
16. Centrifuge at 8,000 x g for 2 minutes at room temperature.
17. The 1.5 ml tube contains your purified viral RNA.

The flow chart describes the steps for purification of viral RNA from 250 µl samples.
(Scale-Up)



V Troubleshooting

Problem	Cause	Solution
Low RNA yield	Poor quality fluid biological samples (Often RNA is degraded by RNases in the starting material)	・ Always use fresh samples for purification. ・ Samples should be processed immediately. If necessary, add RNase inhibitor to the sample and ensure appropriate storage conditions up to the processing. ・ Repeated freezing and thawing should be avoided.
	Incomplete lysis	・ To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Proteinase K and NIRV Extraction Buffer.
	Fragmentation of RNA	・ Do not vortex more than 15 seconds. Be especially careful after incubation.
	RNase contamination	・ Follow the general guideline for handling of RNA. ・ Use RNase-free consumables.
	Low concentration of virus in the sample	・ Follow the optional protocol on page 7 to Scale-Up.
Genomic DNA contamination	Samples containing cells	・ If possible, pellet the cells using centrifugation, and use supernatant for isolation of viral RNA. ・ If DNA-free RNA is required, digest the eluate with RNase-free DNase.

Related products

- Distilled Water, Deionized, Sterile (Code No. 316-90101, 318-90105)
- DNase I (RNase free) (Code No. 314-08071)
- RNase Inhibitor (Code No. 315-08121)
- Collection Tube (Code No. 319-08141, 315-08143)

VI Acknowledgment

This product was developed based on the results of joint research with Professor Yoshitomo Morinaga and Associate Professor Hideki Tani of Department of Microbiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama.

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