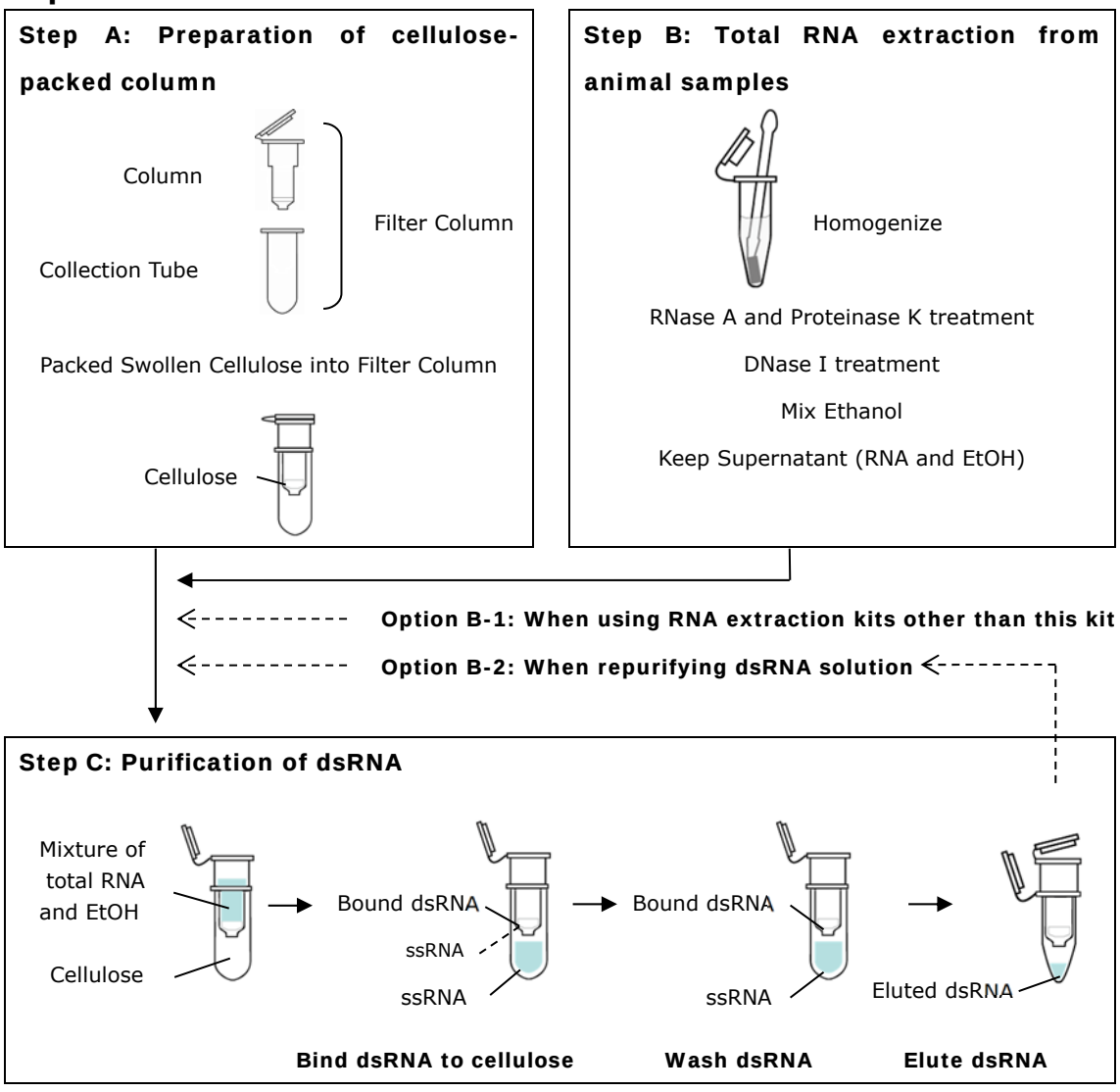
ISOVIRUS II

Code No. 312-09091
Manual Ver. 1

Introductions:

The ISOVIRUS II is designed to purify viral double-stranded RNA (dsRNA) for animal samples, including cultured cells and mammalian tissues. Cellulose column chromatography allows selective capture of dsRNA. Purified dsRNA is suitable for use in downstream applications such as RNA sequencing, etc.

Experimental Overview.



Safety Data Sheet:

The Safety Data Sheets (SDSs) are available on our website at **www.nippongene.com**.

Kit Contents:

The materials provided are sufficient for 20 preparations.

Component	Quantity	Storage
Proteinase K	500 μl x 1	-20˚C
DNase I (RNase free)	1,000 units x 1	-20˚C
10 x DNase I Buffer	1.2 ml x 1	-20˚C
RNase A	30 μl x 1	Room temp.*1
VR Extraction Buffer CT1	10 ml x 1	Room temp.
VR Extraction Buffer 2	2 ml x 1	Room temp.*2
2×STE Buffer	7 ml x 1	Room temp.
Filter Column	20 pieces x 1 bag	Room temp.*3
Swollen Cellulose	10 tubes x 2 boxes	Room temp.*4
VR Wash Buffer	40 ml x 2	Room temp.*4
VR Elution Buffer	4 ml x 1	Room temp.

Reagents required but not supplied: ethanol and nuclease-free water.

*1 For long-term storage, store the RNase A at 4°C or -20°C.

*2 If the VR Extraction Buffer 2 contains a precipitate, warm the buffer in a +65°C water bath and mix to dissolve the precipitate.

*3 A Filter Column consists of a column pre-inserted into a collection tube.

*4 Swollen Cellulose and VR Wash Buffer contain ethanol. Always keep buffer bottles tightly closed.

Required Materials Not Included:

Pipettors and pipette tips

Micro-pestles (designed for manual homogenization of tissue samples)

1.5 ml microcentrifuge tubes

Centrifuge tubes for cell culture

Centrifuge for spin down

Microcentrifuge capable of centrifuging 13,000 x g at 4°C
Heat blocks or water baths set to 37°C and 65°C
Vortex mixer
Ice
Ethanol (95-100 %)
Water (RNase free)
Liquid nitrogen, if necessary

Method

Step A: Preparation of cellulose-packed column

For each cellulose-packed column, prepare one Filter Column (a column in a collection tube) and one Swollen Cellulose vial (~800 µl).

1. Resuspend the Swollen Cellulose in its tube by through vortexing. Transfer the Swollen Cellulose from the vial to the Filter Column directly or by pipetting using a pipette tip with the tip cut off.

2. Incubate for 1-3 minutes at room temperature (leave the cap open) to drain the buffer from cellulose by gravity. If the caps are closed tight, buffer may not be drained out.

3. Cap the column and spin down for 3 seconds. Remove the column from the collection tube and discard flow-through. Re-insert the column in the same collection tube.
(Stopping Point) Cellulose-packed columns can be stored at 4˚C for at least one week. Close the cap to avoid drying.

4. Slowly apply 500 µl VR Wash Buffer into the column. Do not disturb the packed cellulose surface.

5. Incubate for 3-5 minutes at room temperature (leave the cap open) to drain the buffer.

6. Cap the column and spin down for 3 seconds. Discard flow-through and re-insert the column in the collection tube.

7. Slowly apply 900 µl VR Wash Buffer into the column.

8. Incubate for 3-5 minutes at room temperature (leave the cap open) to drain the buffer.

9. Close the cap to avoid drying. The cellulose-packed columns can be stored at 4˚C for up to one day.

Proceed to Step C. Part 1: Bind dsRNA to cellulose.

Step B: Total RNA extraction from animal samples

Samples should be flash frozen or rapidly processed after harvest to remain RNA intact. Sample input amount: up to 100 mg tissue, 50 mg tissue (with high content), or 10^7 cultured mammalian cells.

1. Transfer animal sample into a clean 1.5 ml microcentrifuge tube.
[For cultured mammalian cells, cells should be pelleted in a centrifuge tube and all culture medium removed prior to use of this kit.]

2. Add 500 µl of VR Extraction Buffer CT1 to sample and grind tissue using a micro-pestle. For maximal RNA recovery, use a micro-pestle that fits the conical contour of a 1.5 ml microcentrifuge tube.
[For cells, lyse cells by repeated pipetting. Be sure the cell pellet is completely resuspended in the VR Extraction Buffer CT1.]
(Note) Incomplete homogenization will reduce the yield of recovered RNA.

3. Add 1 µl RNase A to the tube, mix by inversion.

4. Add 20 µl Proteinase K to the tube, mix by gentle inversion. Do not vortex.

5. Incubate the tube at 65˚C for 15 minutes. During incubation, gently mix every 5 minutes. Do not vortex.

6. Add 100 µl VR Extraction Buffer 2 to the tube, mix by inversion.

7. Incubate for 3 minutes at room temperature.

8. Add 60 µl 10 x DNase I Buffer and 30 units DNase I (RNase free). Mix by inversion.

9. Incubate the tube at 37˚C for 15 minutes. During incubation, gently mix every 5 minutes. Do not vortex.

10. Add 35 µl Water (RNase free) and 35 µl 2x STE Buffer.

11. Add 143 µl 95–100% ethanol to the tube to obtain a final ethanol concentration of 16%, close the cap, and mix by inversion.

12. Incubate on ice for 10 minutes.

13. Centrifuge for 5 minutes at 13,000 x g at 4˚C to pellet cellular debris. Cellular debris may float on the liquid surface.

14. Transfer supernatant (~900 µl) to a clean microcentrifuge tube, avoiding the debris. If the volume of supernatant is less than expected, an additional centrifugation step of 13,000 x g will be required.

15. Close the cap to avoid drying. The supernatant containing 16% ethanol can be stored at 4˚C for up to 4 hours or at -20˚C for up to 16 hours.

Proceed to Step C. Part 1: Bind dsRNA to cellulose.

Step C. Part 1: Bind dsRNA to cellulose

For efficient binding of dsRNA to the cellulose-packed column from Step A, ensure the supernatant from Step B contains 16% ethanol prior to loading onto the column.

1. Spin down the cellulose-packed column from **Step A** for 3 seconds. Remove the column from the collection tube and discard flow-through. Re-insert the column in the same collection tube.

2. Apply the entire supernatant (~900 µl) from **Step B** to the column.

3. Incubate for 3-15 minutes at room temperature (leave the cap open) to drain the buffer from cellulose by gravity. Depending on the sample concentration, the buffer may not be drained out even after 15 minutes. In that case, proceed to the next spin step.
(Note) If cellulose begins to float by air bubbles, flick the tube, open-and-close the cap of the column a few times, or tap the tube lightly on the desk to settle the cellulose into the column.

4. Cap the column and spin down for 3-5 seconds. Proceed to the next step to collect the flow-through.

5. Remove the column from the collection tube and collect the buffer from the collection tube by pipetting. Re-insert the column into the same collection tube and re-apply the collected buffer to the column.

6. Incubate for at least 3 minutes at room temperature (leave the cap open).

7. Cap the column and spin down for 3-5 seconds. Proceed to the next step to collect the flow-through.

8. Remove the column from the collection tube and collect the buffer from the collection tube. Re-insert the column into the same collection tube and re-apply the collected buffer to the column.

9. Incubate for at least 3 minutes at room temperature (leave the cap open).

10. Cap the column and spin down for 3-5 seconds. Discard flow-through and re-insert the column in the collection tube.

Proceed to Step C. Part 2: Wash dsRNA.

Step C. Part 2: Wash dsRNA

Wash 3 times by adding VR Wash Buffer to remove any residual single-strand RNA and DNA on the cellulose-packed column.

1. Apply 800 µl VR Wash Buffer into the column from **Part 1 of Step C** to wash the bound dsRNA.
(Note) If cellulose begins to float by air bubbles, flick the tube, open-and-close the cap of the column a few times, or tap the tube lightly on the desk to settle the cellulose into the column.

2. Incubate for 5 minutes at room temperature (leave the cap open).

3. Cap the column and spin down for 3 seconds. Discard flow-through and re-insert the column in the collection tube.

4. Apply 800 µl VR Wash Buffer into the column.
(Note) If cellulose begins to float, to allow the cellulose to settle, flick the tube and so on.

5. Incubate for 5 minutes at room temperature (leave the cap open).

6. Cap the column and spin down for 3 seconds. Discard flow-through and re-insert the column in the collection tube.

7. Apply 800 µl VR Wash Buffer into the column.
(Note) If cellulose begins to float, to allow the cellulose to settle, flick the tube and so on.

8. Incubate for 5 minutes at room temperature (leave the cap open).

9. Cap the column and spin for 5 seconds. Discard flow-through and the collection tube.

10. Insert the column into a clean 1.5 ml microcentrifuge tube.

Proceed to Step C. Part 3: Elute dsRNA.

Step C. Part 3: Elute dsRNA

To elute dsRNA, nuclease-free water (not included) with the pH 8.0-8.5 or VR Elution Buffer (slightly alkaline solution containing 1 mM EDTA) in this kit could be used. Do not use acidic water.

1. Apply 50 µl VR Elution Buffer or neutral nuclease-free water to the center of column from **Part 2 of Step C** so that the elution buffer spreads throughout the cellulose.
(Note) To increase the concentration, the amount of elution buffer could be reduced up to 25 µl.

2. Incubate for 5 minutes at room temperature.
(Note) Whether the cap is open or closed does not affect the results.

3. Spin down for 3 seconds. Proceed to the next step to collect the flow-through.

4. Remove the column and collect the buffer from the tube by pipetting. Re-insert the column into the same tube and re-apply the collected buffer to the column.

5. Incubate for 3 minutes at room temperature.

6. Spin down for 3 seconds. Proceed to the next step to SAVE the flow-through.

7. Remove the column and collect the buffer from the tube by pipetting. Transfer the collected buffer into a new separate tube and save as first eluted dsRNA solution.
(Note) The eluted dsRNA solution can be stored at -20˚C.

8. Re-insert the column into the same tube and apply 50 µl VR Elution Buffer or neutral nuclease-free water to the center of column.
(Note) To increase the concentration, the amount of elution buffer could be reduced up to 25 µl.

9. Incubate for 5 minutes at room temperature.

10. Spin down for 3 seconds. Proceed to the next step to collect the flow-through.

11. Remove the column and collect the buffer from the tube by pipetting. Re-insert the column into the same tube and re-apply the collected buffer to the column.

12. Incubate for 3 minutes at room temperature.

13. Spin down for 3 seconds. Proceed to the next step to SAVE the flow-through.

14. Remove the column and collect the buffer from the tube by pipetting. Transfer the collected buffer into a new separate tube and save as second eluted dsRNA solution.
(Note) The eluted dsRNA solution can be stored at -20˚C.

15. Combine the first eluted dsRNA solution with the second eluted dsRNA solution to make a total volume of 100 µl dsRNA solution. Alternatively, save each 50 µl of eluted dsRNA solution separately.
(Note) The concentration of second eluted dsRNA solution may be lower than that of the first eluted dsRNA solution.
(Optional) The eluted dsRNA can be exchanged solution buffers using ISOSPIN PCR Product (not included, Code No. 315-08001).

Option B-1: When using RNA extraction kits other than this kit

Instead of Step B, it is possible to combine option B-1 with a nucleic acid solution or total RNA solution extracted from animal samples using RNA extraction kit, such us ISOGEN (Code No. 311-02501), ISOSPIN Cell & Tissue RNA (Code No. 314-08211) and so on.

1. Transfer ~100 µl nucleic acid solution (does not need to be 100 µl) into a clean 1.5 ml microcentrifuge tube.

2. Add 500 µl of VR Extraction Buffer CT1 and 1 µl RNase A to the tube, mix by inversion.

3. Incubate for 5 minutes at room temperature.

4. Add 20 µl Proteinase K to the tube, mix by gentle inversion. Do not vortex.

5. Incubate the tube at 37˚C for 15 minutes. During incubation, gently mix every 5 minutes. Do not vortex.

6. Add 100 µl VR Extraction Buffer 2 to the tube, mix by inversion.

7. Add 60 µl 10 x DNase I Buffer and 30 units DNase I (RNase free). Mix by inversion.

8. Incubate the tube at 37˚C for 15 minutes. During incubation, gently mix every 5 minutes. Do not vortex.

9. Add 35 µl Water (RNase free) and 35 µl 2x STE Buffer.

10. Add 143 µl 95–100% ethanol to the tube to obtain a final ethanol concentration of 16%, close the cap, and mix by inversion.

11. Incubate on ice for 10 minutes.

12. Centrifuge for 5 minutes at 13,000 x g at 4˚C.

13. Transfer supernatant to a clean microcentrifuge tube.

14. Close the cap to avoid drying.
The supernatant containing 16% ethanol can be stored at 4˚C for up to 4 hours or at -20˚C for up to 16 hours.

Proceed to Step C. Part 1: Bind dsRNA to cellulose.

Option B-2: When repurifying dsRNA solution

In case of contamination of single-stranded RNA in the eluted dsRNA solution from Step C, you can start with Option B-2 to repurify it.

1. Transfer the dsRNA solution (does not exceed to 350 µl) into a clean 1.5 ml microcentrifuge tube. Add nuclease-free water to bring the volume to 350 µl.

2. For every 350 µl dsRNA solution, add 350 µl of 2x STE Buffer to bring the volume to 700 µl.

3. Add 133 µl 95–100% ethanol to the tube to obtain a final ethanol concentration of 16%, close the cap, and mix by vortex.

4. Close the cap to avoid drying.
The mixture containing 16% ethanol can be stored at 4˚C for up to 4 hours or at -20˚C for up to 16 hours.

Proceed to Step C. Part 1: Bind dsRNA to cellulose.

Troubleshooting:

Observation	Potential cause	Suggested action
Low dsRNA yield.	Large sample input amounts reduce lysis efficiency.	Reduce amount of starting material.
	Elution buffer has acidic pH.	Use the VR Elution Buffer in the kit.
	Inappropriate ethanol concentration.	For dsRNA to bind to the column, a final ethanol concentration of 16% is needed.

References:

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- Ryo Okada, Eri Kiyota, Hiromitsu Moriyama, Toshiyuki Fukuhara, Tomohide Natsuaki (2015) *J Gen Plant Pathol* **81**: 103-107
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Companion Products:

Item	Code No.
Distilled Water, Deionized, Sterile	318-90105
Water, Nuclease free	314-09291
ISOSPIN PCR Product	315-08001
DNase I (RNase free)	317-09661
RNase A (100 mg/ml)	318-06391
ISOVIRUS (for plant, fungi)	310-08811
ISOGEN	311-02501
ISOSPIN Cell & Tissue RNA	314-08211

(Note) This product was developed based on the research results of new academic field research "Neo-Virology."

For Research Use Only. Not for diagnostic procedures.

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