ISOSPIN Plant RNA

Manual (Ver. 05)

Code No. 310-08171

NIPPON GENE CO., LTD.

I Description

The ISOSPIN Plant RNA enables a rapid isolation of highly pure total RNA within 1 hour from plant tissues.

II Kit components

Component	(50 preps)	Note
PT Extraction Buffer (for plant)	30 ml x 1	
PT Binding Buffer (for plant)	40 ml x 1	(includes ethanol)*
PT Wash1 Buffer	40 ml x 1	(includes ethanol)*
PT Wash2 Buffer	40 ml x 1	(includes ethanol)*
DNase I (RNase free)	2,000 units x 1	
10 x DNase I Buffer	1 ml x 1	
ddWater (RNase free)	1 ml x 8	
Spin Column (a Spin Column and Collection Tube set)	50 sets	

* Keep the buffer bottles tightly closed after use.

III Storage conditions

DNase I (RNase free) should be stored at -20°C. The remaining kit components can be stored at room temperature (15-25°C).

IV Precautions

- The kit is intended research use only.

V Protocol

Material not supplied

- Micropipette
- Pipette tips
- Pestle
- 1.5 mL microcentrifuge tubes
- Microcentrifuge (4°C)

Material, as necessary

- liquid nitrogen
- Assist Buffer for ISOSPIN Plant RNA (Code No.315-08501)*

* See Optional Protocol on Page 6.

Standard Protocol

1. Prepare a fresh or frozen tissues **Sample** (20-100 mg) into a 1.5 mL microcentrifuge tube.

   Note)

   ・To avoid a degradation of RNA, keep samples cold and work quickly in this Step.

   ・Ensure tissues samples are homogenized in Step 2 immediately after harvest or are frozen immediately in liquid nitrogen.

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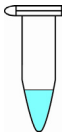

2. Add 600 µl of **PT Extraction Buffer (for plant)**, and homogenize tissue samples with a Pestle.

   Note) To avoid clogging, mix it well.

3. Centrifuge for 10 min at 13,000 x g at 4°C. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

   Note)

   ・For samples with high lipid content, transfer the supernatant carefully without disturbing the floating lipid.

   ・For samples with a high content of extracellular material, centrifuge the supernatant obtained in Step 3 for 10 min at 13,000 x g at 4°C again. Transfer the supernatant to a new tube without disturbing the pellet.

4. Add an equal amount of **PT Binding Buffer (for plant)**, and mix well by inverting. Spin down lightly.

   Example) Add 550 µl of PT Binding Buffer (for plant) to 550 µl of the supernatant.

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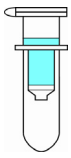

5. Prepare a **Spin Column** (a Spin Column and Collection Tube set). Apply 600 µl of the **Mixture** to the Spin Column. Centrifuge for 1 min at 13,000 x g at 4°C.

6. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

7. Apply the remaining Mixture to the Spin Column, and repeat Step 5 and 6.

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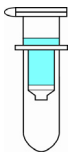

8. Apply 500 µl of **PT Wash1 Buffer** to the Spin Column.

9. Centrifuge for 1 min at 13,000 x g at 4°C.

10. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

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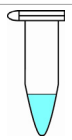

11. Prepare 100 µl of a **DNase I solution** from the following into a new 1.5 mL microcentrifuge tube.

10 x DNase I Buffer	10 µl
DNase I (RNase free)	30 units
ddWater (RNase free)	up to 100 µl

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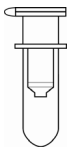

12. Add 100 µl of the DNase I solution to the Spin Column. Incubate for 15 min at room temperature.

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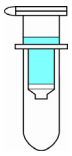

13. 300 µl of **PT Wash1 Buffer** to the Spin Column.

14. Centrifuge for 1 min at 13,000 x g at 4°C.

15. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

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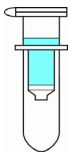

16. Add 600 µl of **PT Wash2 Buffer** to the Spin Column.

17. Centrifuge for 2 min at 13,000 x g at 4°C. Discard the flow-through and the Collection Tube.

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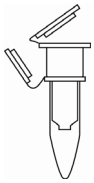


18. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

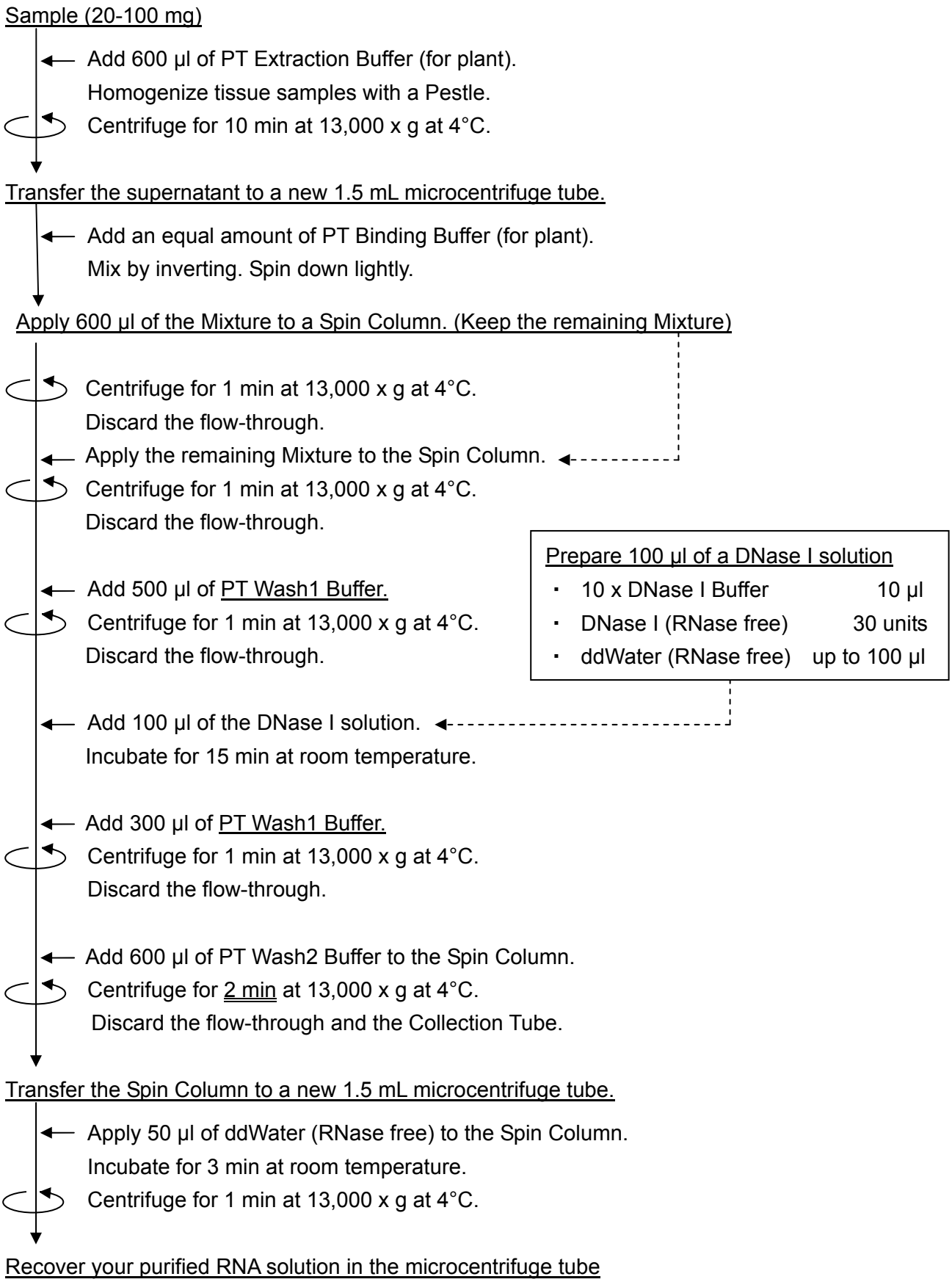
19. Add 50 µl of ddWater (RNase free) to the center of the Spin Column. Incubate for 3 min at room temperature.

20. Centrifuge for 1 min at 13,000 x g at 4°C.

21. Recover your purified RNA solution in the microcentrifuge tube.

Note) The RNA solution can be used directly or stored at -80°C for long term storage.

Simplified Standard Protocol



Optional Protocol

Assist Buffer for ISOSPIN Plant RNA (Code No.315-08501), an Assist Buffer 1 and 2 set, is required besides this kit. This optional protocol using the Assist Buffers is beneficial for samples that are unable to isolate pure and high yield RNA in the Standard Protocol from.

1. Prior to use, prepare 600 µl of an **Extraction Solution**; To 500 µl of **PT Extraction Buffer (for plant)**, add 60 µl of **Assist Buffer 1** and 40 µl of **Assist Buffer 2**, and mix.

   Note)

   ・The Extraction Solution must be prepared immediately before use because a mixture of Assist Buffer 1 and Assist Buffer 2 cannot be stored.

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2. Prepare a fresh or frozen tissues **Sample** (5-100 mg) into a new 1.5 mL microcentrifuge tube.

   Note)

   ・It is recommended to increase samples gradually.

   ・If your samples have a high content of extracellular material, the following prewashing might be more effective. Grind tissue samples lightly with a Pestle in 300 µl of TE within a minute. Centrifuge for 10 min at 13,000 x g at 4°C, and discard the supernatant.

   ・To avoid a degradation of RNA, keep samples cold and work quickly in this Step.

   ・Ensure tissues samples are homogenized in Step 2 immediately after harvest or are frozen immediately in liquid nitrogen.

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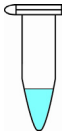

3. Add 600 µl of the Extraction Solution, and homogenize tissue samples with a Pestle.

   Note) To avoid clogging, mix it well.

4. Centrifuge for 10 min at 13,000 x g at 4°C. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

5. Centrifuge the supernatant for 10 min at 13,000 x g at 4°C again. Transfer the supernatant to a new tube.

6. Add an equal amount of **PT Binding Buffer (for plant)**, and mix well by inverting. Spin down lightly.

   Example) Add 550 µl of PT Binding Buffer (for plant) to 550 µl of the supernatant.

7. Centrifuge the **Mixture** for 10 min at 13,000 x g at 4°C. Transfer the supernatant to a new tube.

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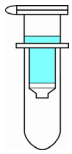

8. Prepare a **Spin Column** (a Spin Column and Collection Tube set). Apply 600 µl of the supernatant obtained in Step 7 to the Spin Column. Centrifuge for 1 min at 13,000 x g at 4°C.
   Note) If the supernatant has not completely passed through the column after centrifugation, centrifuge again.

9. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

10. Apply the remaining supernatant to the Spin Column, and repeat Step 8 and 9.

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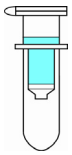

11. Apply 500 µl of **PT Wash1 Buffer** to the Spin Column.

12. Centrifuge for 1 min at 13,000 x g at 4°C.

13. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

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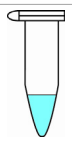
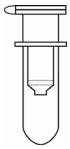


14. Prepare 100 µl of a **DNase I solution** from the following into a new 1.5 mL microcentrifuge tube.

10 x DNase I Buffer	10 µl
DNase I (RNase free)	30 units
ddWater (RNase free)	up to 100 µl

↓



15. Add 100 µl of the DNase I solution to the Spin Column. Incubate for 15 min at room temperature.

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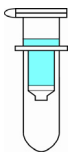

16. 300 µl of **PT Wash1 Buffer** to the Spin Column.

17. Centrifuge for 1 min at 13,000 x g at 4°C.

18. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

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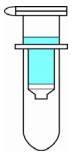

19. Add 600 µl of **PT Wash2 Buffer** to the Spin Column.

20. Centrifuge for 2 min at 13,000 x g at 4°C. Discard the flow-through and the Collection Tube.

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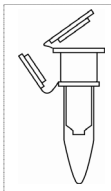

21. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

22. Add 50 µl of ddWater (RNase free) to the center of the Spin Column. Incubate for 3 min at room temperature.

23. Centrifuge for 1 min at 13,000 x g at 4°C.

24. Recover your purified RNA solution in the microcentrifuge tube.
    Note) The RNA solution can be used directly or stored at -80°C for long term storage.

Simplified Optional Protocol

Assist Buffer for ISOSPIN Plant RNA (Code No. 315-08501) is required besides this kit.

Prepare 600 µl of an Extraction Solution

- PT Extraction Buffer (for plant) 500 µl
- Assist Buffer 1 60 µl
- Assist Buffer 2 40 µl

Sample (20-100 mg)

← Add 600 µl of the Extraction Solution.

Homogenize tissue samples with a Pestle.

Centrifuge for 10 min at 13,000 x g at 4°C.

Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

Centrifuge for 10 min at 13,000 x g at 4°C.

Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

← Add an equal amount of PT Binding Buffer (for plant), and mix by inverting.

Centrifuge for 10 min at 13,000 x g at 4°C.

Apply 600 µl of the supernatant to a Spin Column. (Keep the remaining supernatant)

Centrifuge for 1 min at 13,000 x g at 4°C.

Discard the flow-through.

← Apply the remaining supernatant to the Spin Column.

Centrifuge for 1 min at 13,000 x g at 4°C.

Discard the flow-through.

Prepare 100 µl of a DNase I solution

- 10 x DNase I Buffer 10 µl
- DNase I (RNase free) 30 units
- ddWater (RNase free) up to 100 µl

← Add 500 µl of PT Wash1 Buffer.

Centrifuge for 1 min at 13,000 x g at 4°C.

Discard the flow-through.

← Add 100 µl of the DNase I solution.

Incubate for 15 min at room temperature.

← Add 300 µl of PT Wash1 Buffer.

Centrifuge for 1 min at 13,000 x g at 4°C.

Discard the flow-through.

← Add 600 µl of PT Wash2 Buffer to the Spin Column.

Centrifuge for 2 min at 13,000 x g at 4°C.

Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

← Apply 50 µl of ddWater (RNase free) to the Spin Column.

Incubate for 3 min at room temperature.

Centrifuge for 1 min at 13,000 x g at 4°C.

Recover your purified RNA solution in the microcentrifuge tube.

VI Troubleshooting

Problem	Cause and countermeasure
Low yield	RNA degradation caused by incomplete homogenization or lysis of samples. Ensure tissue sample is excised as quickly as possible and homogenized efficiently.
RNA degradation	Immediately after harvesting tissues samples, homogenize in Extraction Buffer or frozen in liquid nitrogen.
	Store frozen samples at -70°C or below.
	Use RNase-free solutions and tubes for dissolving RNA.
DNA contamination	Perform a DNase treatment on recovered purified RNA solution if you have not performed Step 11 and Step 12.
Variable absorbance values	Low purity: Extracted RNA is contaminated with impurities such as polysaccharides. See the following countermeasures against Contamination with impurities.
Contamination with impurities	Reduce the amount of samples for Extraction buffer in Step 1 to lyse efficiently.
	Centrifuge the supernatant obtained in Step 3 for 10 min at 13,000 x g at 4°C again. Transfer the supernatant to a new tube without disturbing the pellet and the float.
Still have trouble	Try the optional protocol on page 6.

The information in the descriptions of the products may be changed without prior notification.

NIPPON GENE CO., LTD.

If you have any questions, please contact us by web form.
http://www.nippongene.com/siyaku/