ISOSPIN Cell & Tissue RNA

Manual (Ver. 02)

Code No. 314-08211

NIPPON GENE CO., LTD.

I Description

The ISOSPIN Cell & Tissue RNA enables a rapid isolation of highly pure total RNA within 1 hour from animal tissues or cultured cells.

II Kit components

Component	(50 preps)	Note
PT Extraction Buffer (for tissues)	30 ml x 1	
C Extraction Buffer (for cells)	30 ml x 1	
PT Binding Buffer (for tissues & cells)	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

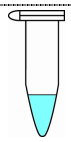
- Micropipette
- Pipette tips
- Pestle
- 1.5 mL microcentrifuge tubes
- Microcentrifuge*

* Centrifugation of the protocol can be performed at 4°C or 25°C.

Material, as necessary

- liquid nitrogen

Cultured Cells RNA Extraction Protocol



1. Add 600 µl of **C Extraction Buffer** to **Sample (up to 3 x 10^6 cultured cells)**, and lyse cells by pipetting.

2. Vortex more than 30 sec.
   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.

4. Add an equal amount of **PT Binding Buffer**, and mix well by inverting. Spin down lightly.
   Example) Add 550 µl of PT Binding Buffer to 550 µl of the supernatant.

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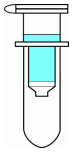

5. Prepare a **Spin Column** (a Spin Column and Collection Tube set). Apply 600 µl of the **Mixture** to the Spin Column. Centrifuge for 1 min at 13,000 x g at 4°C.

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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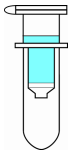

8. Apply 500 µl of **PT Wash1 Buffer** to the Spin Column.

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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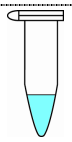

11. Prepare 100 µl of a **DNase I solution** from the following into a new 1.5 mL microcentrifuge tube.

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

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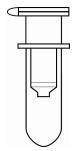

12. Add 100 µl of the DNase I solution to the Spin Column. Incubate for 15 min at room temperature.

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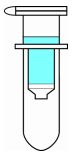

13. 300 µl of **PT Wash1 Buffer** to the Spin Column.

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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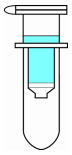

16. Add 600 µl of **PT Wash2 Buffer** to the Spin Column.

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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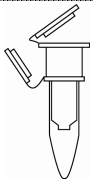


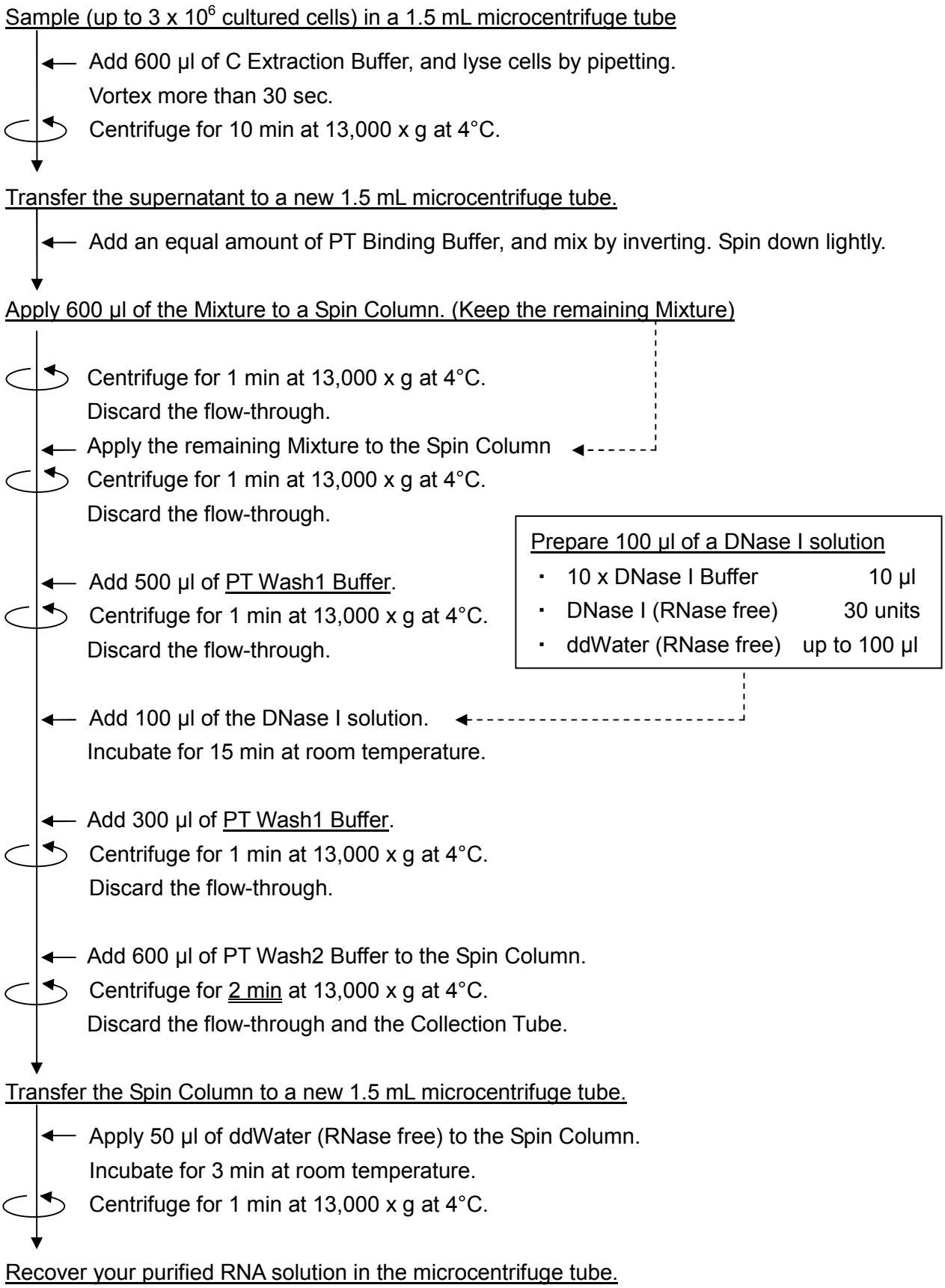
18. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

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.

Cultured Cells RNA Extraction Simplified Protocol



Animal Tissues RNA Extraction Protocol

1. Prepare a fresh or frozen tissues **Sample** (up to 10 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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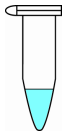

2. Add 600 µl of **PT Extraction Buffer**, and homogenize tissue samples with a Pestle. Vortex more than 30 sec.

   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**, and mix well by inverting. Spin down lightly.

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

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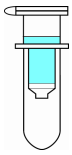

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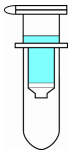

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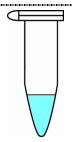

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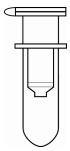

12. Add 100 µl of the DNase I solution to the Spin Column. Incubate for 15 min at room temperature.

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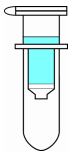

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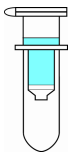

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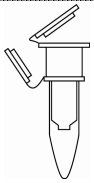

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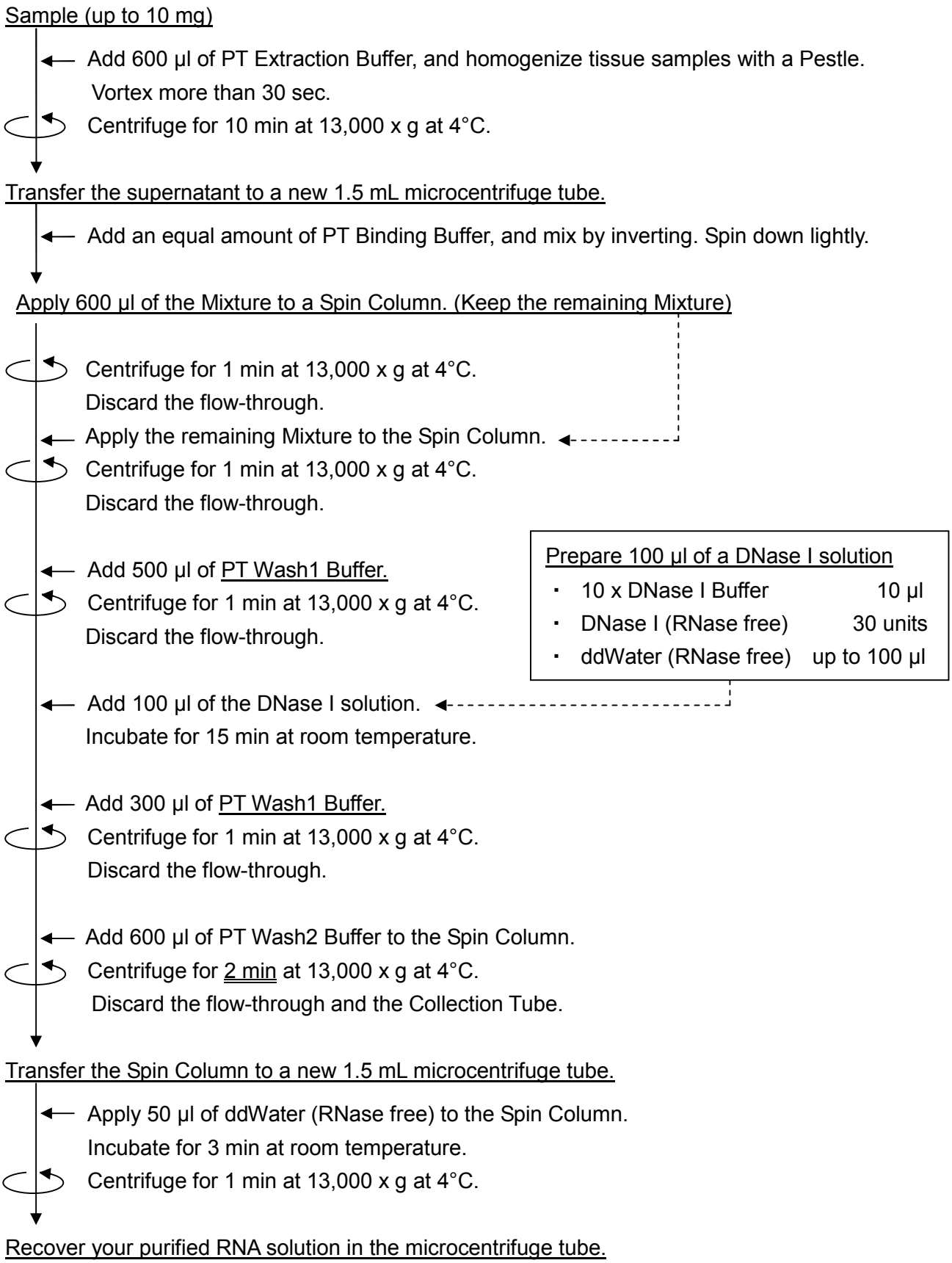


VI Troubleshooting

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.

VII Data

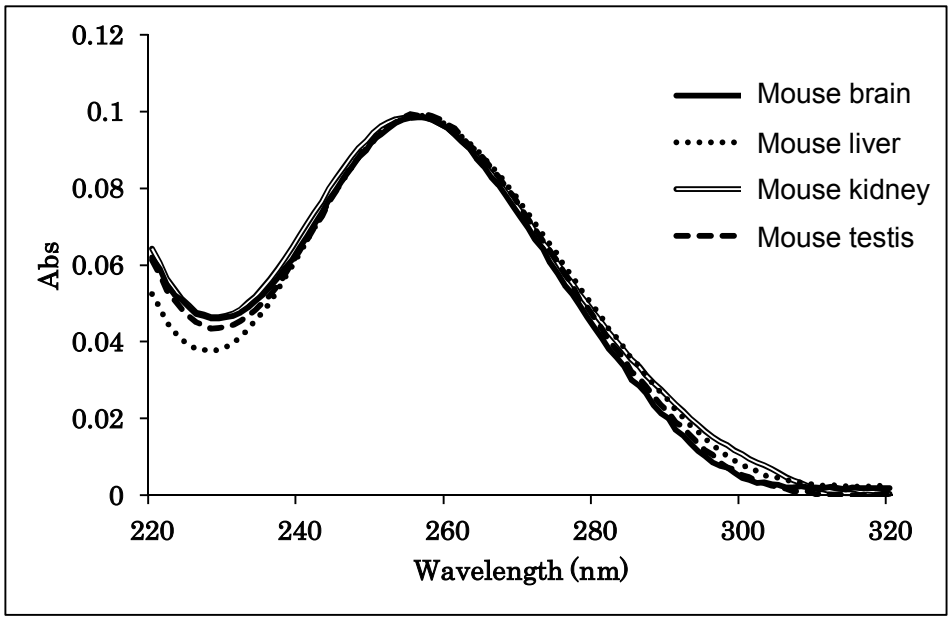
Technical Information

RNA Binding Volume	100 μg
Column Volume	900 μl

Expected yields

Sample	Expected yields
HeLa cell	15 μg RNA/10^6 cells
Jurkat cells	10 μg RNA/10^6 cells
Vero cell	15 μg RNA/10^6 cells
Mouse brain	1.0 μg RNA/mg tissue
Mouse liver	3.5 μg RNA/mg tissue
Mouse kidney	3.0 μg RNA/mg tissue
Mouse testis	1.5 μg RNA/mg tissue

Absorption spectra of RNA



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/