

Reagent for RNA Extraction

ISOGEN with Spin Column

Manual (4th Edition)

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NIPPON GENE CO., LTD.

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I Product description

The ISOGEN with Spin Column enables a rapid isolation of highly pure total RNA within 1 hour by utilizing the combination of ISOGEN and a spin column containing silica-gel membranes. Efficient extraction of total RNA from a variety of samples can be achieved with the use of ISOGEN in a single-step liquid-phase separation. Also, our unique spin column allows purification of high-quality RNA in the presence of chaotropic ions using the Boom method.

■ Features

- Total RNA can be extracted with high purity.
- No alcohol precipitation is necessary.
- High-quality RNA can be purified from plant samples with high impurity contents.

II Product content

| Contents | (for 10 extractions) | (for 50 extractions) |
|-------------|----------------------|----------------------|
| ISOGEN | 10 ml | 50 ml |
| IsoWash I | 9 ml | 30 ml |
| IsoWash II | 7 ml | 30 ml |
| TE (pH8.0) | 1 ml | 5 ml |
| Spin Column | 10 sets | 50 sets |

III Storage

Store at +2°C to +10°C

- Use up this product as soon as possible after opening (within 6 months from the purchase date).
- This product is shipped at room temperature. Upon receipt, store the ISOGEN at 2 to 10°C in the dark. The remaining kit components can be stored at room temperature.

IV Precautions

- This product is intended for research use and not for other purposes. Also, this product should be handled only by persons having a basic knowledge about reagents.
- ISOGEN contains phenol (a “non-medical deleterious substance”) and should be handled with great care.
- Wear appropriate protective gear when handling (gloves, goggles, etc.).
- Avoid inhaling vapor. Use in well ventilated area.
- If the product enters the eyes or adheres to the skin, wash with large amounts of water for at least 15 min and consult a doctor.
- Handle this product in accordance with the descriptions in this manual.
- We are not responsible for problems caused by failure to follow the instructions in this manual.
- For more information, refer to the Safety Data Sheet (SDS) available on our website at www.nippongene.com

V Protocol

<Required reagents, instruments and the like not included in this kit>

- Sample (fresh tissue, frozen tissue, culture cell, plant, etc.)
- chloroform
- 70% ethanol
- Isopropanol
- Micropipette
- Pipette tips
- 1.5 ml microtubes
- Centrifuge (4°C)

<Reagents, instruments and the like to be prepared, as necessary>

- RNase-free water
- Liquid nitrogen
- Polytron homogenizer or glass-Teflon homogenizer
- Water bath (50°C)

We recommend the use of clear polypropylene tubes for homogenizing sample with ISOGEN. Make sure that the tubes can withstand centrifugation at 12,000 x g with ISOGEN (phenol) and chloroform.

< Handling procedures >

1. Homogenize sample in 1 ml of ISOGEN (25-100 mg of tissue sample per 1 ml of ISOGEN).

Note) To maximize yield and purity of total RNA, efficient homogenization of the starting material is a significant requirement.

- Ensure tissue samples are homogenized in ISOGEN immediately after harvest or are frozen immediately in liquid nitrogen.
- Homogenization of tissue samples can be carried out efficiently using a Polytron homogenizer at high speed for 2-3 min.
- Before weighing tissue samples, pre-weigh a tube filled with an adequate volume (1-5 ml) of ISOGEN and set the zero point of balance. As soon as the freshly harvested or frozen sample is placed in the pre-weighed tube, weigh the total mass to determine the weight of the tissue. Immediately disrupt and homogenize the sample. Adjust to the appropriate volume by adding ISOGEN and resuspend the homogenate.
- The homogenate can be stored overnight at 4°C or at least one year at -20°C or -70°C. When thawing the homogenate, incubate for 5 min at 37-40°C.

Animal tissues: homogenize tissue samples with a glass-Teflon homogenizer or Polytron homogenizer using up to 100 mg tissue per 1 ml of ISOGEN. When homogenizing impurity-rich tissue samples (such as liver and spleen), use 25-50 mg tissue per 1 ml of ISOGEN.

For samples with high lipid content, centrifuge the homogenate at 12K x g for 5 min at 4°C to remove lipid material. Transfer the supernatant carefully without disturbing the floating lipid layer.

Cultured cells: add ISOGEN immediately after removing culture medium. Pretreatment before the addition of ISOGEN, such as with trypsin or washing cells, is not recommended as it may contribute to RNA degradation.

A: Adherent cells: remove culture medium from the culture dish, add at least 1 ml of ISOGEN per 3.5 cm dish or 6-well plate (10 cm²), and lyse cells completely by pipetting. Use the amount of ISOGEN based on the area of the culture dish and not on cell number.

B: Suspension cells: Collect cells by centrifuging first and remove supernatant (culture medium). Then add at least 1 ml of ISOGEN per 1×10^7 cells and lyse cells by pipetting (if the number of cells is unknown, add 10 times volume of ISOGEN to the pellet).

Plant tissues: use up to 100 mg tissue in 1ml of ISOGEN. When homogenizing impurity-rich samples, use 25 mg of sample per 1 ml of ISOGEN.

Freeze sample in liquid nitrogen and pulverize. Homogenize the sample in ISOGEN preheated at 50°C and incubate for 10 min in a water bath. After incubation, shake vigorously and centrifuge at 12K x g for 10 min at 4°C. Transfer 1 ml of the supernatant to a new tube carefully without disturbing the pellet.

When processing samples with high impurity content such as seed and bulb, freeze the resulting

supernatant at -80°C for 1 hour and thaw by letting stand at room temperature. Centrifuge and transfer 1 ml of the supernatant to a new tube carefully without disturbing the pellet.

2. After incubating the homogenate from Step 1 for 5 min at room temperature, add 0.2 times volume of chloroform per ISOGEN used ($200\ \mu\text{l}$ of chloroform per 1 ml of ISOGEN). Shake the mixture vigorously for 15 sec and store for 2 min at room temperature. Centrifuge the sample at $12\text{K} \times g$ for 15 min at 4°C .

Note)

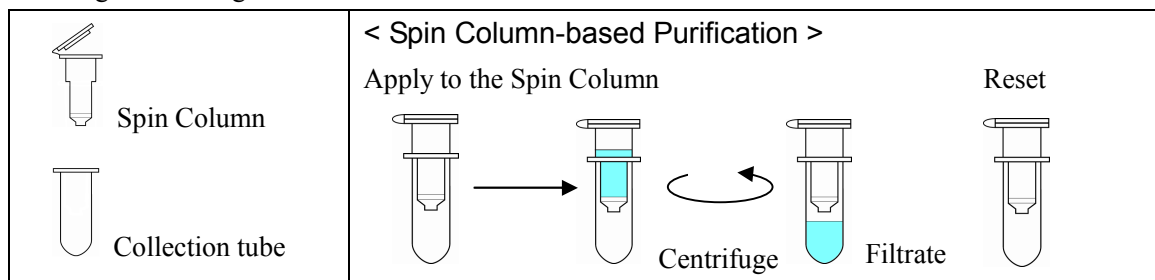
- Do not use chloroform that contains isoamyl alcohol (such as CIA 24:1).
- The mixture separates into a lower organic phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the upper aqueous phase while most of DNA and proteins remain in the interphase or organic phase.
- The volume of the aqueous phase is about 60% of the volume of ISOGEN used in Step 1. In case the volume of the aqueous phase is extremely low or is below 30% of the volume of ISOGEN used, shake the mixture vigorously and re-centrifuge. If this still does not resolve the issue, it is recommend to repeat procedure with smaller amount of starting material.

3. Transfer $400\ \mu\text{l}$ of the colorless upper aqueous phase to a new tube.

Note) Transfer carefully without disturbing the interphase and organic phase.

4. Add an equal amount ($400\ \mu\text{l}$) of 70% ethanol to the aqueous solution from Step 3, and mix by inverting.

5. Load the entire volume of the mixture from Step 4 ($800\ \mu\text{l}$) onto a Spin Column. Close the lid, and centrifuge at $12\text{K} \times g$ for 15 sec at 4°C .



6. Transfer $700\ \mu\text{l}$ of the filtrate to a new microtube and mix with $200\ \mu\text{l}$ of isopropanol. This mixture will be used in Step 8.

Place the Spin Column back into the collection tube and proceed to Step 7.

Note)

- In this step RNA binds to the membrane in the Spin Column; all of the RNA may not bind depending on the presence of impurities derived from the starting materials.
- Do not transfer the entire volume of filtrate. Carefully collect from the top surface without aspirating impurities left in the bottom (sometimes not visible).

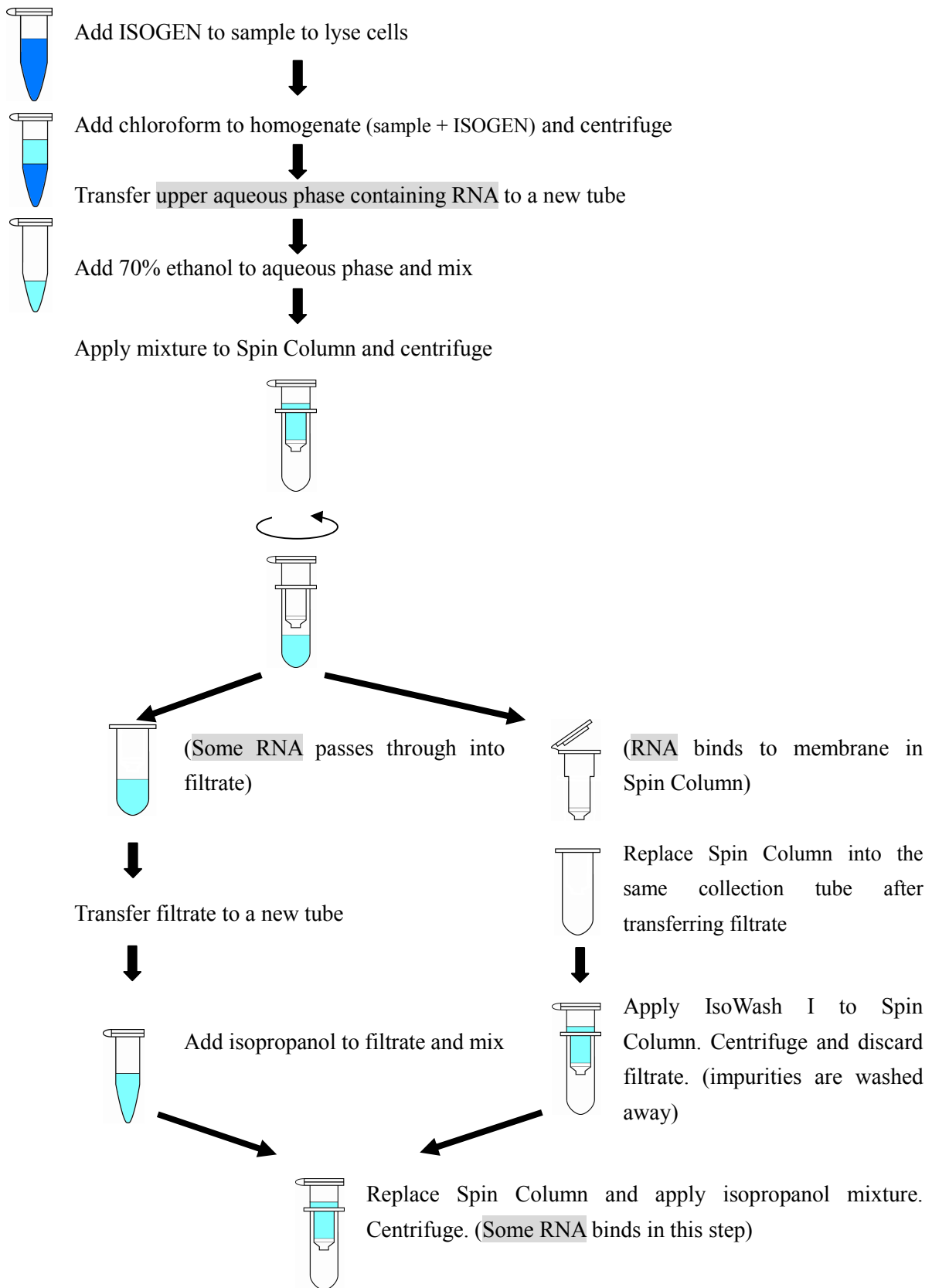
- Optional DNase treatment may be performed on the Spin Column after performing this step (See troubleshooting/options in p.9.)
7. Apply 500 µl of IsoWash I to the Spin Column from Step 6. Centrifuge at 12 K x g for 15 sec at 4°C. Discard the filtrate.
 8. Apply the entire volume of the mixture from Step 6 (filtrate + isopropanol) to the Spin Column from Step 7. Centrifuge at 12 K x g for 15 sec at 4°C. Discard the filtrate.
 Note) RNA that passed through into the filtrate in Step 6 is bound to the membrane during this step. If large precipitates are seen on the membrane, repeat the wash with 300 µl of IsoWash I, centrifuge at 12K x g for 15 sec at 4°C, and discard the filtrate.
 9. Apply 300 µl of IsoWash II to the Spin Column from Step 8. Centrifuge at 12 K x g for 15 sec at 4°C.
 10. Apply 300 µl of IsoWash II to the Spin Column from Step 9. Centrifuge at 12 K x g for 1 min at 4°C. Discard the collection tube containing the filtrate.
 11. Place the Spin Column from Step 10 into a new 1.5 ml microtube. Apply 20-50 µl of TE or RNase-free water and leave standing for 1 min.
 12. Centrifuge at 12 K x g for 1 min at 4°C at room temperature to elute the RNA.

The table below lists typical yields of RNA from various materials.

| Sample | Expected Yield | |
|----------------------------------|--------------------|------------------------------------|
| <u>Animal tissues</u> (Mouse) | Brain | 0.5 µg RNA/mg tissue |
| | Liver | 3 µg RNA/mg tissue |
| | Kidney | 2 µg RNA/mg tissue |
| | Testis | 2 µg RNA/mg tissue |
| <u>Cultured cells</u> | Jurkat cells | 4 µg RNA/1 x 10 ⁶ cells |
| | HeLa cells | 4 µg RNA/1 x 10 ⁶ cells |
| <u>Plant tissues</u> | Cabbage sprout | 0.3 µg RNA/mg tissue |
| | Rice leaf | 0.3 µg RNA/mg tissue |
| | Soybean seed | 0.5 µg RNA/mg tissue |
| | Corn seed | 0.5 µg RNA/mg tissue |
| | Cabbage seed | 0.3 µg RNA/mg tissue |
| | Tulip bulb | 0.2 µg RNA/mg tissue |
| | Unhulled rice seed | 0.2 µg RNA/mg tissue |

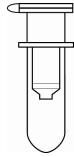
Dilute in a buffer with pH >8.0 to measure absorbance accurately (such as TE, pH8.0).

<Simplified operation flow>



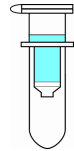
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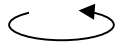


Discard filtrate

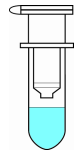
(If large precipitates are seen on the membrane, repeat the wash with IsoWash I)



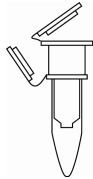
Apply IsoWash II and centrifuge (15 sec)



Apply IsoWash II and centrifuge (1 min)



Discard filtrate and collection tube.



Replace Spin Column onto a new 1.5 ml microtube



Apply TE or RNase-free water and centrifuge



Elute RNA

VI Troubleshooting

| Problem | Cause and countermeasure |
|-------------------------------|---|
| Low yield | RNA degradation caused by incomplete homogenization or lysis of samples occurred in cells that are not in contact with ISOGEN solution. Ensure tissue sample is excised as quickly as possible and homogenized efficiently. See note in Step 1 in p.4. |
| RNA degradation | Immediately after harvesting tissue samples, homogenize with ISOGEN or freeze in liquid nitrogen. |
| | Store frozen samples at -70°C or below. |
| | Use RNase-free solutions and tubes for dissolving RNA. |
| DNA contamination | Increase the amount of ISOGEN to be added to sample. |
| | Do not use samples containing an organic solvent, strong buffer, salt or alkaline solution. |
| | Do not collect the entire aqueous phase after phase separation. Leave a small volume of the aqueous phase right above the interphase to avoid aspirating DNA from the lower phase. |
| | Perform DNase treatment. See options below. |
| Variable absorbance values | Measurement of absorbance values in water may result in variable and inaccurate values. Measure in a buffer with pH 8.0 or higher, such as TE (pH 8.0), for reproducible results. |
| | Low purity: Extracted RNA is contaminated with impurities such as polysaccharides. See the following countermeasures against Low purity and Contamination with impurities. |
| Low purity | Insufficient amount of ISOGEN causes insufficient lysis of samples. Repeat procedure with half the amount of starting material or with twice volume of ISOGEN. |
| | Contamination with lipids, proteoglycans, polysaccharides, etc. See the following countermeasures against Contamination with impurities. |
| Contamination with impurities | Remove lipids and insoluble materials by centrifuging the homogenate (12 K x g, 10 min). |
| | For samples with high impurity content such as seed and bulb, freeze the homogenate and then centrifuge to remove the impurities. |

<Options>

- **DNase treatment for RT-PCR applications:** Perform DNase treatment of RNA samples with Deoxyribonuclease (RT Grade) for Heat Stop (Code No. 312-05951).

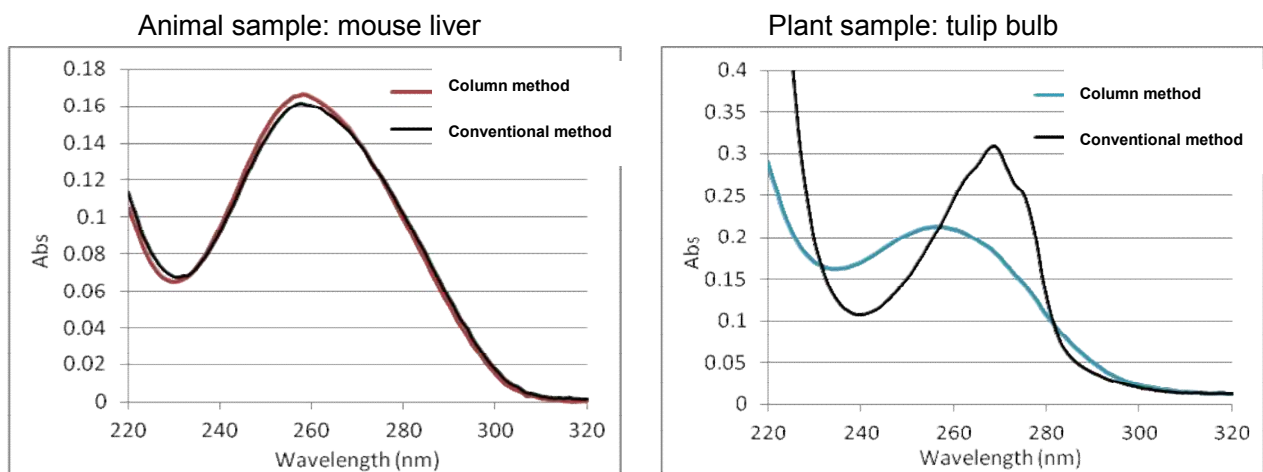
- **DNase treatment on Spin Column:** This can be performed right after Step 6 (before the wash with IsoWash I in Step 7) with DNase I (RNase free) (Code No. 314-08071). Prepare the DNase solution

beforehand as below, apply 100 µl of the DNase solution to the Spin Column, and leave standing for 15 min at room temperature. Thereafter, proceed to Step 7.

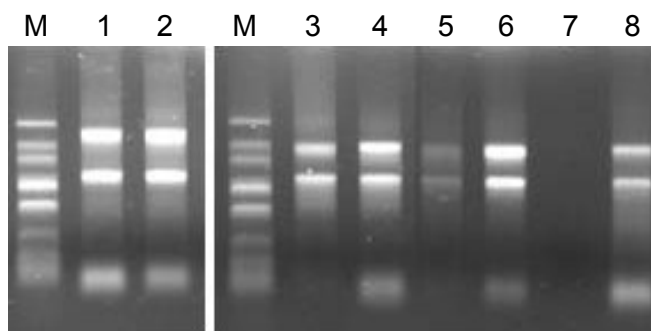
DNase solution (not supplied and prepare before use): Dilute 30 units of DNase I (RNase free) in 10 µl of the Reaction Buffer (10 x Buffer) supplied with the enzyme in a tube. Bring up to 100 µl with RNase-free water and mix by gently pipetting.

VII Data

In accordance with the protocol in this manual, RNA was extracted from mouse liver (50 mg) and frozen pulverized plant samples (25 mg each) using this product and the absorption spectra were measured. In parallel, RNA was extracted using the conventional method (RNA was purified by isopropanol precipitation after extracted using ISOGEN).



Further, 0.5 µg of RNA calculated based on the absorbance of respective RNA solutions was electrophoresed in formaldehyde denatured agarose gel.



- Lane 1: Mouse liver (Conventional method)
- Lane 2: Mouse liver (Column method)
- Lane 3: Cabbage (Conventional)
- Lane 4: Cabbage (Column)
- Lane 5: Tulip bulb (Conventional)
- Lane 6: Tulip bulb (Column)
- Lane 7: Unhulled rice seed (Conventional)
- Lane 8: Unhulled rice seed (Column)

M: RNA Ladder (0.125 - 6.0 kb)
1% gel Agarose S. EtBr staining

The results with animal tissue samples showed RNA with high purity equivalent to that of obtained using the conventional method was obtained. (similar results with cultured cell samples were also obtained).

In comparison of the two methods using the plant samples, the column method using the ISOGEN with Spin Column produced RNA of higher purity (seen especially in bulbs and unhulled rice seeds) without impurities that have absorbance near at 230 nm, such as polysaccharides. This can be confirmed from the result of electrophoresis.

NIPPON GENE CO., LTD.

If you have any questions, please contact us by web form.

<http://www.nippongene.com/>

• The information in the descriptions, specifications and the prices of the products may be changed without prior notification.

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