Cat. # 9186

For Research Use

TaKaRa

Magnosphere™ UltraPure mRNA Purification Kit

Product Manual

v202004Da

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I. Description

The Magnosphere UltraPure mRNA Purification Kit is designed to isolate and purify polyA$^+$ RNA from total RNA derived from cultured cells or animal tissues. The Magnosphere Oligo-dT Beads in this kit are magnetic particles developed by JSR Life Sciences Corporation that enable high-purity isolation of polyA$^+$ RNA with an extremely low rate of ribosomal RNA (rRNA) contamination. The beads have a uniform particle size and super paramagnetism, allowing efficient isolation by magnets. They also leave no residual magnetization, offering excellent redispersibility. These properties make it possible to quickly and efficiently isolate polyA$^+$ RNA without the need for centrifugation. In addition, compared with conventional magnetic particles, this product has lower nonspecific adsorption of impurities on the particle surface, allowing specific and efficient recovery of polyA$^+$ RNA. In contrast to purification kits that use non-magnetic-particle carriers, this kit is well-suited for purifying polyA$^+$ RNA from trace amounts of total RNA.

With this kit, typically, high-quality polyA$^+$ RNA with low rRNA contamination can be recovered with a yield of 0.5% to 2% from 5 to 50 μg of total RNA. This kit is ideal for preparing polyA$^+$ RNA samples with low rRNA contamination, which are required for applications including NGS sequencing, cDNA synthesis with random primers, etc.

II. Components (for 20 reactions)*1

(1)	Magnosphere Oligo-dT Beads*2	400 μl
(2)	2X Binding Buffer	7 ml
(3)	Wash Buffer	24 ml
(4)	RNase-free Water	1.4 ml

Magnosphere Oligo-dT Beads

Magnetic beads 1% (w/v) Suspension

10 mg/ml	$Oligo(dT)_{30}$ Immobilized Magnetic Particles
25 mM	Tris-HCl
150 mM	NaCl
0.05%	Tween 20
0.09%	NaN_3*3

*1 5 to 50 μg of total RNA can be used per reaction.

*2 The Magnosphere Oligo-dT Beads are manufactured by JSR Life Sciences Corporation.

*3 NaN_3 may react with metals to form highly explosive metal azides. When disposing, flush with a large volume of water.

III. Materials Required but not Provided

- Magnetic stand
  e.g., Magnetic Stand (6 tubes) (Cat. #5328)*
- Thermal cycler (65℃, for heat denaturation)
- Heat block (80℃, for incubation)
- Various low-adsorption tubes
  0.2 ml tubes; e.g.0.2ml Single-Tube Dome Cap (Cat. #NJ204)
  1.5 ml tubes; e.g.1.5 ml SnapLock Microtube, Non-Sterile, MaxyClear, Maxymum Recovery (AXYGEN Cat. #MCT-150-L-C)
- Spectrophotometer
  When assaying the concentration of minute quantities, the use of a NanoDrop series microvolume spectrophotometer (ThermoFisher Scientific) is recommended.
- Agilent 2100 Bioanalyzer (Agilent Technologies)
- Reagents needed for Agilent 2100 Bioanalyzer, Agilent RNA 6000 Nano Assay, and Agilent RNA 6000 Pico Assay (Agilent Technologies)

* Not available in all geographic locations. Check for availability in your area.

IV. Storage

4℃
Note: Do not freeze Magnosphere Oligo-dT Beads

V. Sample Preparation (total RNA)

This kit is designed for polyA$^+$ RNA purification from total RNA. To recover high-purity full-length polyA$^+$ RNA, it is critical to use high-purity total RNA samples. Thus, it is important to inhibit cellular RNase activity and to prevent RNase contamination from external sources, such as equipment and solutions used. Exercise meticulous care while preparing RNA. For example, avoid RNase contamination from perspiration or saliva, use clean disposable gloves, and dedicate a laboratory bench exclusively for RNA preparation.

[Equipment]

Use disposable plastic labware whenever possible. General glassware should be treated according to the following protocol prior to use:

(1) Treat glassware with 0.1% diethyl pyrocarbonate (DEPC) solution at 37℃ for 12 hours or longer.

(2) Autoclave (120℃, 30min) to remove residual DEPC.

Only use equipment (plastic and glass) that are dedicated exclusively to RNA experiments.

[Solutions]

Prepare all reagents for experiments in glassware that has been dry-heat sterilized (180℃, 60min) and use sterile purified water or ultrapure water that has been treated by 0.1% DEPC and autoclaved. Only use solutions, sterile purified water, and ultrapure water that are dedicated exclusively for RNA experiments.

[total RNA sample preparation]

Highly purified total RNA can be prepared by methods such as the acid guanidinium thiocyanate phenol chloroform method (AGPC method). For extractions from cells or tissues, the use of RNAiso Plus (Cat. #9108/9109) or NucleoSpin RNA (Cat. #740955.10/.50/.250) enables quick preparation of high-purity total RNA.

VI. Precautions for Use

[Magnosphere Oligo-dT Beads]

1. If clumps are visible in the reagent solution, disperse well by pipetting up and down before use.
2. Avoid using this product under strong acidic conditions.
3. Although this product contains 0.09% sodium azide as a preservative, take care not to introduce bacterial contamination after opening.
4. The magnetic particles may precipitate during long-term refrigeration. Before use, incubate the beads at 37℃ and then mix well to suspend the particles adequately. Suspend by pipetting or tapping; avoid vigorous vortexing.
5. When the amount of polyA$^+$ RNA recovered is low, RNA content cannot be accurately measured by effect of residual trace reagents. The residual reagents are removed from polyA$^+$ RNA by phenol-chloroform extraction and ethanol precipitation. Ethanol precipitation using Dr. GenTLE® Precipitation Carrier (Cat. #9094) enables recovery of highly concentrated polyA$^+$ RNA.

VII. Protocol

Place the reagents at the appropriate temperatures listed below and set instruments to the required temperatures.

The 2X Binding Buffer may develop precipitates during storage. Incubate at 37℃ and mix gently. Make sure the precipitates are completely dissolved before use.

- Magnosphere Oligo-dT Beads : Room temperature
- 2X Binding Buffer : 37℃
- Wash Buffer and RNase-free Water : Room temperature
- Thermal cycler : 65℃
- Heat block : 80℃

Note :

- Make sure to use low adsorption tubes for each step.
- Protocol A) and B) below are for the treatment of 5 to 50 μg of total RNA. When treating more than 50 μg of total RNA in a single reaction, scale up the amount of magnetic particles (Magnosphere Oligo-dT Beads) accordingly. For example, use 5 times the volume of magnetic particles when treating 250 μg of total RNA.

  The volume of the 2X Binding Buffer used in Step A)-6 to resuspend the magnetic particles should also be increased to equal the volume of the total RNA solution. However, do not increase the volumes specified in the protocol below for the buffers used in other steps (e.g., 2X Binding Buffer, Wash Buffer, and RNase-free Water).

A) Preparation of magnetic particles (Magnosphere Oligo-dT Beads)

1. Allow the magnetic particles (Magnosphere Oligo-dT Beads) to equilibrate to room temperature, and mix well to obtain a uniform suspension. Place 20 μl into a 1.5 ml tube.
2. Add 100 μl of the 2X Binding Buffer* that has been incubated at 37℃ to the magnetic particles.
   * Make sure to use 2X Binding Buffer.
3. Set the tube in the magnetic stand, and allow to stand for at least 1min.
4. Remove the supernatant. Do not aspirate the magnetic particles.
5. Repeat the procedures in Steps A)-2 through 4 to wash the magnetic particles.
6. Remove the tubes from the magnetic stand, and resuspend the magnetic particles with 2X Binding Buffer in a volume equal (50 to 100 μl) to that of the total RNA solution that will be used in Step B)-1.

B) PolyA$^+$ RNA purification

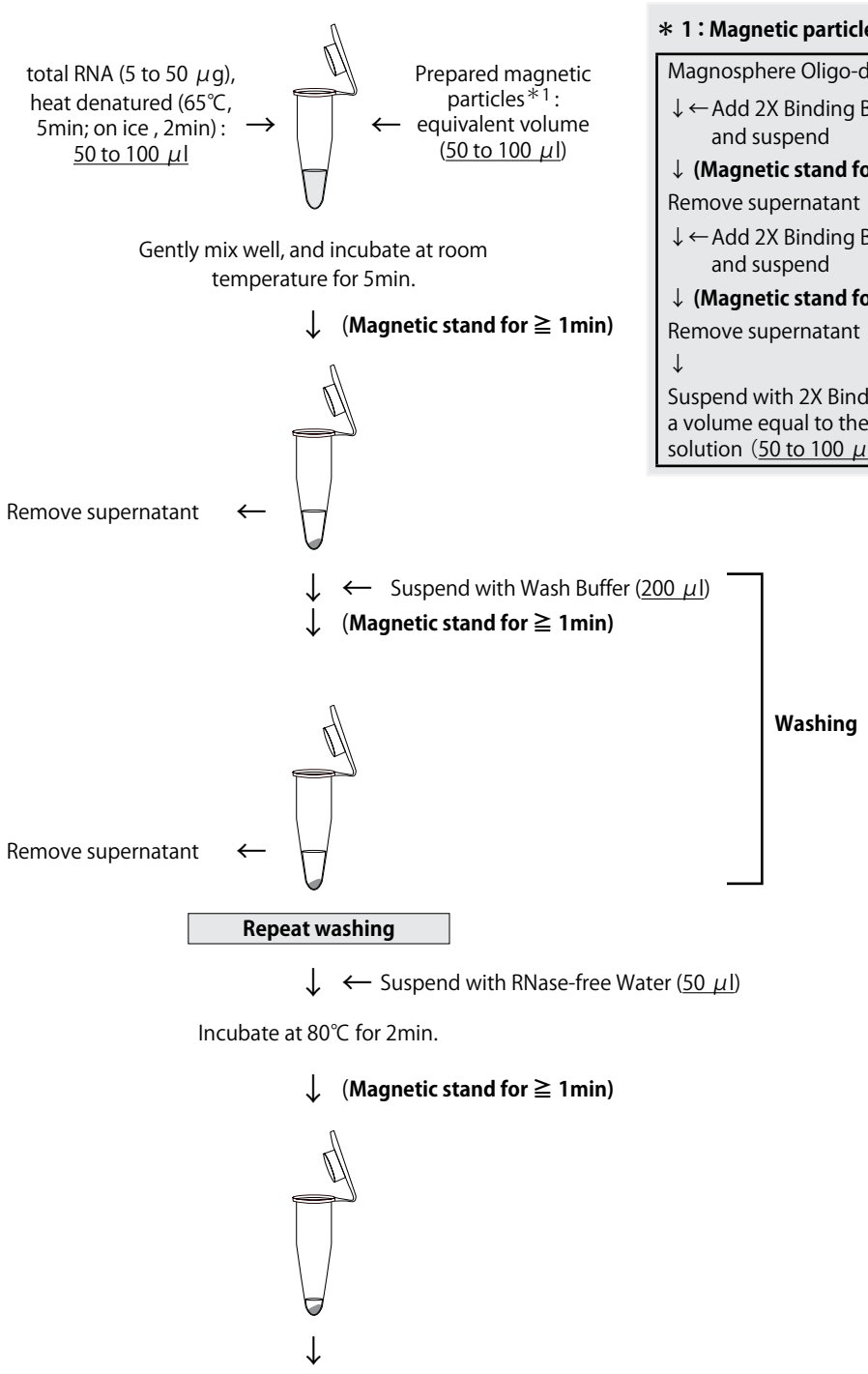
1. Prepare 50 to 100 μl of total RNA (5 to 50 μg) in a 0.2 ml tube.
2. Heat denature the RNA at 65℃ for 5min in a thermal cycler, and then place the tube on ice for 2min.
3. Add the entire volume of the denatured RNA to the magnetic particle suspension prepared in Step A)-6, and mix gently.
4. Let stand at room temperature for 5min.
   * Do not place in the magnetic stand at this step.
5. Set the tubes to the magnetic stand, and let sit for at least 1min.
6. Remove the supernatant while making sure not to aspirate the magnetic particles.
7. Remove the tubes from the magnetic stand, and suspend the magnetic particles with 200 μl of Wash Buffer.
8. Set the tubes in the magnetic stand, and let them sit for at least 1min.
9. Remove the supernatant. Do not aspirate any of the magnetic particles.
10. Repeat the procedures in Steps B)-7 through 9 to wash the magnetic particles.
11. Suspend the magnetic particles completely with 50 μl of RNase-free Water.
12. Incubate the magnetic particles in a heat block at 80℃ for 2min.
13. Set the tubes in the magnetic stand, and let them sit for at least 1 min.
14. While making sure not to aspirate the magnetic particles, transfer the supernatant to a new 0.2 ml tube.
    * Do not discard the magnetic particles.
15. Add 50 μl of 2X Binding Buffer to the recovered supernatant and mix. Keep at room temperature until ready to use in Step B)-20.

16. Suspend the remaining magnetic particles from Step B)-14 with 200 μl of Wash Buffer.
    ＊ Make sure to use Wash Buffer.
17. Set the tubes in the magnetic stand, and let them sit for at least 1min.
18. Remove the supernatant while making sure not to aspirate the magnetic particles.
19. Repeat Steps B)-16 through 18.
20. Heat denature the solution (100 μl) prepared in Step B)-15 in a thermal cycler at 65℃ for 5min, and then place on ice for 2min.
21. Add the denatured RNA solution from Step B)-20 (100 μl) to the washed magnetic particles from Step B)-19, and mix to suspend the magnetic particles.
22. Let stand at room temperature for 5min.
    ＊ Do not place in the magnetic stand at this step.
23. Set the tubes in the magnetic stand, and let them sit for at least 1min.
24. Remove the supernatant. Do not aspirate the magnetic particles.
25. Remove the tubes from the magnetic stand, and suspend the magnetic particles with 200 μl of the Wash Buffer.
26. Let the tubes sit in the magnetic stand for at least 1min.
27. Remove the supernatant. Do not aspirate the magnetic particles.
28. Repeat Steps B)-25 through 27 to wash the magnetic particles.
29. Remove the tubes from the magnetic stand, and suspend the magnetic particles with 20 μl of the RNase-free Water.
30. Incubate the tubes in a heat block at 80℃ for 2min.
31. Set the tubes in the magnetic stand, and let sit for at least 1min.
32. While making sure not to aspirate any of the magnetic particles, transfer the supernatant (polyA$^+$ RNA fraction) to a new tube and store at -80℃.

Note : If the complete removal of all trace amounts of DNA is required (e.g., if the samples will be used for RT-PCR), the recovered RNA solution can be treated with Recombinant DNase I (RNase-free) (Cat. #2270A/B).

[Workflow]

- Make sure to use low-adsorption tubes in each process.
- Equilibrate reagents to the appropriate temperature (see Section VII. Protocol) before use.



Recover 50 µl of supernatant (Wash the magnetic particles; do not discard*2)

↓ ← Add 2X Binding Buffer (50 µl) and mix

Heat denature the supernatant at 65℃ for 5min, and then place on ice for 2min.

↓

← Add the denatured RNA solution (100 µl) to the washed magnetic particles*2, and mix to suspend

＊2 Magnetic particle washing

Magnetic particles left behind
↓←Suspend with Wash Buffer (200 µl)
↓ **(Magnetic stand for ≧ 1min)**
Remove supernatant
↓
Repeat the above washing procedure

Incubate at room temperature for 5min.

↓ **(Magnetic stand for ≧ 1min)**

Remove supernatant ←

↓ ← Suspend with Wash Buffer (200 µl)

↓ **(Magnetic stand for ≧ 1min)**

Remove supernatant ←

Washing

Repeat washing

↓ ← Suspend with RNase-free Water (20 µl)

Incubate at 80℃ for 2min.

↓ **(Magnetic stand for ≧ 1min)**

Recover supernatant (polyA$^+$ RNA)

VIII. Performance

- Yield and purity of polyA$^+$ RNA by spectrometry.

  Measure absorbance of A260 and A280 to verify the yield and purity of the purified polyA$^+$ RNA. When polyA$^+$ RNA for spectrometry is only a minute amount, the use of a NanoDrop series microvolume spectrophotometer (ThermoFisher Scientific) is recommended. For NanoDrop series operating procedures, see the instrument manual. If the concentration of the polyA$^+$ RNA is expected to be below the limit of detection of the NanoDrop, use an Agilent 2100 Bioanalyzer (Agilent Technologies) to determine the concentration, or concentrate the sample by ethanol precipitation with Dr. GenTLE Precipitation Carrier as a co-precipitating agent.

- Yield and purity of polyA$^+$ RNA by Agilent 2100 Bioanalyzer.

  The yield and purity of the purified polyA$^+$ RNA can be analyzed with the Agilent 2100 Bioanalyzer and the RNA 6000 LabChip Kit. High-purity polyA$^+$ RNA has an electrophoretic pattern with extremely low ribosomal RNA (see IX. Experimental Examples). An electrophoretic pattern with a low-molecular-weight signal indicates potential RNA degradation. A gradual slope corresponding to a large molecular weight or a disordered electrophoretic pattern suggests a potential contamination with genomic DNA. In this case, DNase I treatment is recommended.

IX. Experimental Examples

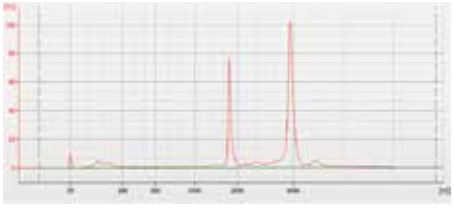
1. Purification of polyA+ RNA from 10 μg and 50 μg of total RNA derived from human HeLa S3 cells

[Methods]

PolyA+ RNA was purified from 10 or 50 μg of total RNA (in a total volume of 100 μl) derived from human HeLa S3 cells with the Magnosphere UltraPure mRNA Purification Kit. The polyA+ RNA obtained were diluted as necessary and analyzed with an Agilent 2100 Bioanalyzer.

[Results]

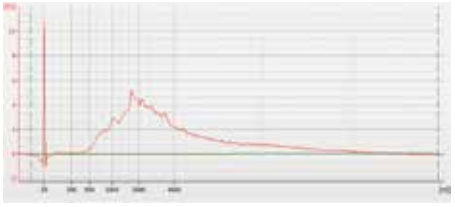
High-purity polyA+ RNA with an extremely low rate of rRNA contamination was prepared. The amount of polyA+ RNA recovered from 10 or 50 μg of total RNA was 0.2 μg and 0.7 μg, respectively (determined by NanoDrop).



[total RNA]

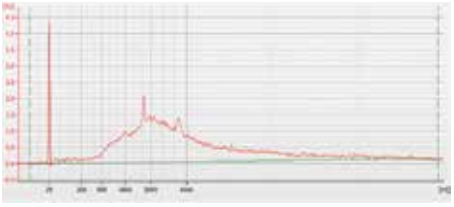
RNA concentration : 137.6 ng/μl
RIN * : 10.0

* RNA Integrity Number, a quality index of RNA measured by the Agilent 2100 Bioanalyzer. The degree of RNA degradation is classified into 10 grades, with higher numbers indicating better quality.



[polyA+ RNA purified from 10 μg of total RNA]

RNA concentration : 821.5 pg/μl
rRNA contamination : 0.7%



[polyA+ RNA purified from 50 μg of total RNA]

RNA concentration : 77.5 ng/μl
rRNA contamination : 1.2%

Figure 1. Results of polyA+ RNA purification (analyzed by Agilent 2100 Bioanalyzer)

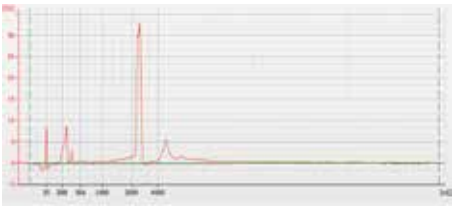
2. Purification of polyA$^+$ RNA from 10 μg of total RNA derived from insect Sf9 cells

[Methods]

PolyA$^+$ RNA from 10 μg of total RNA (in a total volume of 100 μl) derived from the insect Sf9 cells was purified with the Magnosphere UltraPure mRNA Purification Kit. The polyA$^+$ RNA obtained was diluted as necessary and analyzed with an Agilent 2100 Bioanalyzer.

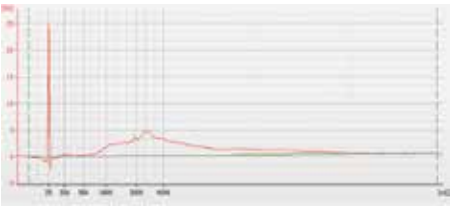
[Results]

High-purity polyA$^+$ RNA with an extremely low rate of rRNA contamination was prepared. The amount of polyA$^+$ RNA recovered was 0.3 μg (determined by NanoDrop).



[total RNA]

RNA concentration : 1.0 ng/μl



[polyA$^+$ RNA]

RNA concentration : 1.0 ng/μl
rRNA contamination : 0.4%

Figure 2. Results of polyA$^+$ RNA purification (analyzed by Agilent 2100 Bioanalyzer)

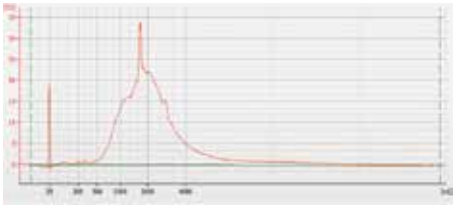
3. Purification of polyA+ RNA from 10 μg of total RNA derived from tobacco BY-2 cells and from 10 μg of total RNA derived from AH109 yeast cells

[Methods]

PolyA+ RNA from 10 μg of total RNA (in a total volume of 50 μl) derived from tobacco BY-2 cells or 10 μg of total RNA derived from AH109 yeast cells was purified with the Magnosphere UltraPure mRNA Purification Kit. The polyA+ RNA obtained were diluted as necessary and analyzed by the Agilent 2100 Bioanalyzer.

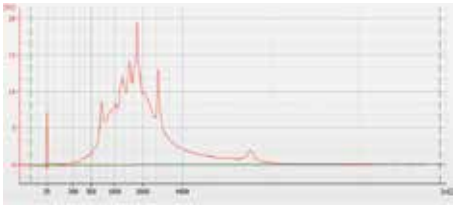
[Results]

High-purity polyA+ RNA with an extremely low rate of rRNA contamination was prepared. The amount of polyA+ RNA recovered was 100 ng from the BY-2 total RNA and 90 ng from the yeast total RNA (determined by NanoDrop).



[PolyA+ RNA derived from tobacco BY-2 cells]

RNA concentration : 3.1 ng/μl
rRNA contamination : 3.2%



[PolyA+ RNA derived from AH109 yeast cells]

RNA concentration : 3.5 ng/μl
rRNA contamination : 6.4%

Figure 3. Results of polyA+ RNA purification (Agilent 2100 Bioanalyzer)

Note: For some species, rRNA contamination (%) can not be accurately determined.

X. Troubleshooting

1. PolyA$^+$ RNA yield is low.
   - The yield may be low when the amount of total RNA sample was significantly less than 5 μg or more than 50 μg.
     → Use an amount of total RNA that falls within the specified range (5 to 50 μg).
   - When the yield was markedly low despite using 5 to 50 μg of total RNA, the cause may be one of the following:
     1) After total RNA was added to the magnetic particles, the mixture was not mixed thoroughly.
        → Mix well in accordance with the protocol.
     2) The magnetic particles were aspirated during supernatant removal in the binding and washing steps.
        → Take great care to avoid aspirating the magnetic particles.
     3) Incubation time in the magnetic stand was short.
        → Allow the tubes to sit in the magnetic stand for the time indicated in the protocol.
     4) RNase contamination occurred during the procedure.
        → Exercise extreme caution when handling RNA samples, reagents, and apparatuses used.
     5) The total RNA starting material was degraded.
        → Perform total RNA extraction again from a fresh tissue or cell sample. Ensure the absence of total RNA degradation before performing polyA$^+$ RNA purification.
     6) The total RNA starting material was contaminated with impurities.
        → Either remove the impurities by purifying the total RNA again or prepare high-purity total RNA again using RNAiso Plus , NucleoSpin RNA, or another RNA purification kit.
     7) Suspension in the RNase-free Water for elution and heating were inadequate.
        → Suspend and heat to an appropriate temperature in accordance with the protocol.
     8) The total RNA were purified from cells or tissues with low polyA$^+$ RNA content.
        → The polyA$^+$ RNA yield varies depending on the type of cell or tissue and the stage of development. Perform a preliminary purification of polyA$^+$ RNA from the target cells or tissues in advance to check the yield if necessary.

2. The purity of polyA$^+$ RNA was poor.
   - When total RNA used exceeded the recommended range, the purity of polyA$^+$ RNA obtained may be low.
     → Use an amount of total RNA that falls within the specified range (5 to 50 μg).
   - A high level of endogenous contaminants in the total RNA may affect polyA$^+$ RNA purity.
     → Either remove contaminants from total RNA before polyA$^+$ RNA purification or prepare high-purity total RNA again using NucleoSpin RNA, etc.
   - rRNA may be difficult to remove from samples of some species.
     → If necessary, perform a preliminary purification of polyA$^+$ RNA.
3. The purified polyA$^+$ RNA was degraded.
   - RNase contamination occurred during the operation.
     → Exercise extreme caution when handling RNA samples, reagents, and apparatuses.
   - The total RNA was degraded.
     → Perform total RNA extraction again from a fresh tissue or cell sample. Ensure the absence of total RNA degradation before performing polyA$^+$ RNA purification.
   - The apparatuses or other equipment used in polyA$^+$ RNA purification were contaminated with RNase.
     → Use RNase-OFF™ (Cat. #9037) or a similar product to remove RNases from apparatuses and working areas.

XI. Related Products

Magnetic Stand（6 tubes）(Cat. #5328)＊
0.2ml Single-Tube Dome Cap（Cat. #NJ204）
NucleoSpin RNA（Cat. #740955.10/.50/.250）
RNAiso Plus（Cat. #9108/9109）
Dr. GenTLE® Precipitation Carrier（Cat. #9094）
Recombinant DNase I (RNase-free)（Cat. #2270A/B）
RNase-OFF™（RNase Decontamination Solution）(Cat. #9037）
Yeast Processing Reagent (for total RNA preparation)（Cat. #9089）
Oligotex-dT30<Super> mRNA Purification Kit（From Total RNA）(Cat. #9086)
PrimeScript™ Double Strand cDNA Synthesis Kit（Cat. #6111A）
cDNA Library Construction Kit（Cat. #6136)＊
In-Fusion® SMARTer® Directional cDNA Library Construction Kit（Cat. #634933）

＊ Not available in all geographic locations. Check for availability in your area.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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