Absolutely RNA Nanoprep Kit

INSTRUCTION MANUAL

Catalog #400753
Revision A

For In Vitro Use Only
400753-12

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (858) 373-6300
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet techservices@agilent.com
World Wide Web www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at *www.stratagene.com*.

Absolutely RNA Nanoprep Kit

CONTENTS

Absolutely RNA Nanoprep Kit

MATERIALS PROVIDED

Materials provided	Quantity[a]
Lysis Buffer	35 ml
β-Mercaptoethanol (β-ME, 14.2 M)[b]	300 µl
RNase-Free DNase I (lyophilized)[c]	2600 U
DNase Reconstitution Buffer	300 µl
DNase Digestion Buffer	1.5 ml
High-Salt Wash Buffer (1.67×)	24 ml
Low-Salt Wash Buffer (5×)	17 ml
Elution Buffer (10 mM Tris-HCl, pH 7.5)	3 ml
RNA-binding nano-spin cups and caps	50 each
2-ml collection tubes (capless)	100

[a] Sufficient reagents are provided to isolate total RNA from 50 samples of 1–10^4 cells each.

[b] Once opened, store at 4°C.

[c] Once reconstituted, store at –20°C.

STORAGE CONDITIONS

Upon Receipt: Store all components at room temperature.

β-Mercaptoethanol: Once opened, store at 4°C.
RNase-Free DNase I: Once reconstituted, store at –20°C.
All Other Components: Once prepared, store at room temperature.

Caution *Guanidine thiocyanate in the Lysis Buffer and High-Salt Wash Buffer is an irritant.*

ADDITIONAL MATERIALS REQUIRED

Sulfolane [Sigma (Catalog #T22209)]
Ethanol
Diethylpyrocarbonate (DEPC)

Revision A

INTRODUCTION

The Absolutely RNA nanoprep kit allows rapid purification of high-quality total RNA from extremely small samples of cultured cells (1–10^4 cells) or cells harvested by laser capture microdissection (LCM).[1] The unique design of the RNA-binding spin cups provided with the Absolutely RNA nanoprep kit allows sample recovery in a very small volume (10 µl), allowing the use of the entire sample in downstream applications without further concentration steps that may lead to degradation of the RNA.

The nanoprep RNA purification method is outlined in Figure 1. The method employs a lysis buffer that contains the chaotropic salt guanidine thiocyanate, a strong protein denaturant, to lyse cells and prevent RNA degradation by ribonucleases (RNases).[2] Following cell lysis, the sample is transferred to a nano-spin cup where the RNA binds to a silica-based fiber matrix.[3] An optional DNase digestion removes contaminating DNA, then a series of washes removes the DNase and other proteins. Highly pure RNA is eluted from the fiber matrix in a low-ionic-strength buffer and is captured in a microcentrifuge tube. The resulting RNA is suitable for use in a multitude of molecular biology applications, including cDNA synthesis, qualitative and quantitative RT-PCR, microarray target preparation, northern blotting and differential display.

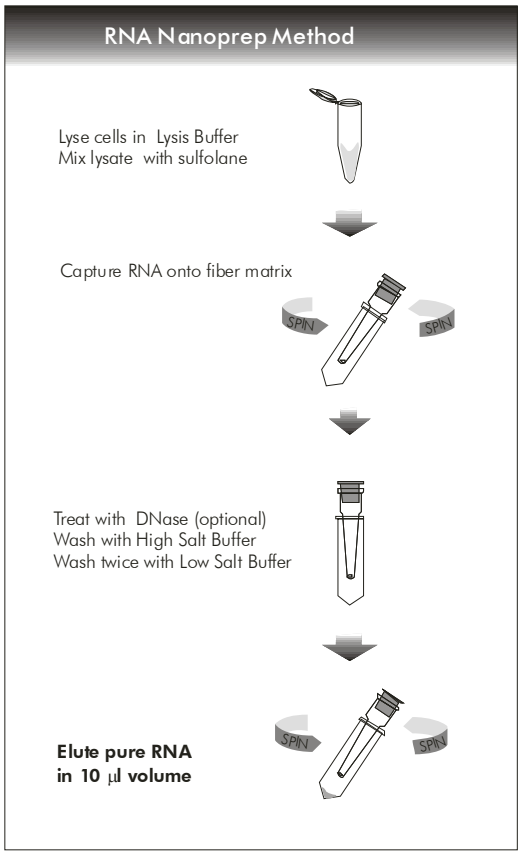

FIGURE 1 Absolutely RNA Nanoprep method.

PREPARING THE REAGENTS

80% Sulfolane

Prepare 80% (v/v) sulfolane by diluting 100% sulfolane with RNase-free water.

Preparation of 5 ml of 80% sulfolane is sufficient for processing 50 RNA preparations (from up to 0.1 ml lysate each). To prepare 5 ml of 80% sulfolane, add 1 ml of RNase-free water to 4 ml of 100% sulfolane.

Notes *100% sulfolane is a solid at room temperature. Prior to diluting the sulfolane, melt by incubating in a 37°C waterbath until liquefied (overnight incubation is convenient for this purpose). The 80% sulfolane solution is a liquid at room temperature, and may be stored at room temperature for at least one month.*

If particulate matter is observed, the sulfolane solution may be filtered using a 0.2 µm nylon filter.

RNase-Free DNase I

Reconstitute the lyophilized RNase-Free DNase I by adding 290 µl of DNase Reconstitution Buffer to the vial. Mix the contents thoroughly to ensure that all the powder goes into solution. Do not introduce air bubbles into the solution. Store the reconstituted RNase-Free DNase I at –20°C.

Note *DNase Reconstitution Buffer is easily added to the vial of DNase with a syringe and needle. Gentle mixing is necessary because the DNase I is very sensitive to denaturation.*

High-Salt Wash Buffer

Prepare 1× High-Salt Wash Buffer by adding 16 ml of 100% ethanol to the bottle of 1.67× High-Salt Wash Buffer.

After adding the ethanol, mark the container as suggested: *[√] 1× (Ethanol Added).* Cap the container of 1× High-Salt Wash Buffer tightly and store at room temperature.

Low-Salt Wash Buffer

Prepare 1× Low-Salt Wash Buffer by adding 68 ml of 100% ethanol to the bottle of 5× Low-Salt Wash Buffer.

After adding the ethanol, mark the container as suggested: *[√] 1× (Ethanol Added)*. Cap the container of 1× Low-Salt Wash Buffer tightly and store at room temperature.

β-Mercaptoethanol

Once opened, store the β-ME at 4°C.

PROTOCOL

RNA Isolation from Cells

The Absolutely RNA Nanoprep Kit is suitable for purification of total RNA from 1–10^4 cells. For RNA preparation from >10^4 cells, we offer the Absolutely RNA total RNA microprep kit (up to 5×10^5 cells, Catalog #400805) and the Absolutely RNA RT-PCR miniprep kit (up to 10^7 cells, Catalog #400800).

1. Add 0.7 µl of β-ME to 100 µl of Lysis Buffer for each sample of up to 1×10^4 cells.

   Caution *The Lysis Buffer contains the irritant guanidine thiocyanate.*

   Notes *Prepare a fresh mixture of Lysis Buffer and β-ME before each use.*

   Cell pellets can be stored at –80°C for future processing; however, homogenizing the cells in Lysis Buffer prior to freezing the pellets is recommended to minimize RNA degradation.

2. Add 100 µl of the Lysis Buffer–β-ME mixture to each cell sample and vortex or pipet the sample repeatedly until homogenized.

   Note *Ensure that the viscosity of the lysate is low. High viscosity causes a decrease in RNA yield and an increase in DNA contamination. The viscosity can be reduced by additional vortexing, pipetting, and/or by increasing the volume of Lysis Buffer (up to 200 µl).*

3. Add an equal volume (usually 100 µl) of 80% sulfolane (stored at room temperature) to the cell lysate and mix thoroughly by vortexing for 5 seconds.

   Note *It is very important to use equal volumes of 80% sulfolane and cell lysate. It is also important to vortex until the lysate and sulfolane are thoroughly mixed.*

4. Transfer this mixture to an RNA-binding nano-spin cup that has been seated within a 2-ml collection tube and snap the cap onto the top of the spin cup.

5. Spin the sample in a microcentrifuge at ≥12,000 × *g* for 60 seconds.

6. Remove and **retain the spin cup** and discard the filtrate. Re-seat the spin cup in the same 2-ml collection tube.

   Note *Up to this point, the RNA has been protected from RNases by the presence of guanidine thiocyanate.*

7. ***Optional DNase Treatment***: This procedure is recommended if DNA-free total RNA is required. If DNA removal is not necessary, omit the DNase treatment and proceed directly to step 8.

    a. Add 300 µl of 1× Low-Salt Wash Buffer to the spin cup. After capping, spin the sample in a microcentrifuge at ≥12,000 × *g* for 60 seconds.

    b. Remove and **retain the spin cup** and discard the filtrate. Re-seat the spin cup in the collection tube, cap the spin cup, and spin the sample in a microcentrifuge at ≥12,000 × *g* for 2 minutes to dry the fiber matrix.

    c. Prepare the DNase solution by gently mixing 2.5 µl of reconstituted RNase-Free DNase I with 12.5 µl of DNase Digestion Buffer for each sample.

        Note *Gentle mixing is necessary because the DNase I is very sensitive to denaturation.*

    d. Add the 15 µl of DNase solution directly onto the fiber matrix inside the spin cup and cap the spin cup.

    e. Incubate the sample at 37°C for 15 minutes.

8. Add 300 µl of 1× High-Salt Wash Buffer to the spin cup, cap the spin cup, and spin the sample in a microcentrifuge at ≥12,000 × *g* for 60 seconds.

    Caution *The High-Salt Wash Buffer contains the irritant guanidine thiocyanate.*

9. Remove and **retain the spin cup,** discard the filtrate, and re-seat the spin cup in the collection tube. Add 300 µl of 1× Low-Salt Wash Buffer. Cap the spin cup and spin the sample in a microcentrifuge at ≥12,000 × *g* for 60 seconds.

10. Perform a second low-salt wash. (Remove and **retain the spin cup,** discard the filtrate, and re-seat the spin cup in the collection tube. Add 300 µl of 1× Low-Salt Wash Buffer. Cap the spin cup and spin the sample in a microcentrifuge at ≥12,000 × *g* for 60 seconds.)

11. Remove and **retain the spin cup**, discard the filtrate, and re-seat the spin cup in the collection tube. Cap the spin cup and spin the sample in a microcentrifuge at ≥12,000 × g for **3 minutes to dry the fiber matrix**.

12. Transfer the spin cup to a fresh 2-ml collection tube.

13. Add 10 µl of Elution Buffer directly onto the fiber matrix inside the spin cup. Cap the spin cup and incubate the sample at room temperature for 2 minutes.

    Note *The Elution Buffer must be added directly onto the fiber matrix to ensure that buffer permeates the entire fiber matrix.*

    The RNA yield may be increased by using Elution Buffer warmed to 60°C.

14. Spin the sample in a microcentrifuge at ≥12,000 × *g* for 5 minutes. This elution step may be repeated to increase the yield of total RNA. The purified RNA is in the eluate in the collection tube. Transfer the eluate to a capped microcentrifuge tube to store the RNA. The RNA can be stored at –20°C for up to one month or at –80°C for long-term storage.

Expected RNA Yields and Quantification

To quantify RNA isolated from 10^2–10^4 cells, a highly sensitive fluorescence-based system (e.g., RiboGreen® RNA quantitation kit, Molecular Probes, Inc.) may be used.

Quantification of RNA isolated from 1–10^4 cells may be achieved using a quantitative RT-PCR (QRT-PCR) assay. A typical QRT-PCR assay probes for a high abundance transcript such as GAPDH and compares the GAPDH levels in experimental samples to those in a standard curve of total RNA to derive the total RNA present in the experimental sample.

The approximate yield obtained using different cell numbers as starting material is given in the table below. Use the numbers presented only as a guideline; expected results will be dependent on the cell type used, growth conditions and other factors.

Cell Number	Yield total RNA
10^4	~100 ng
10^3	~10 ng
10^2	~1 ng
10	≥0.01 ng
1	≥0.01 ng

TROUBLESHOOTING

Observation	Suggestion
RNA is degraded	Use DEPC-treated or radiation-sterilized plasticware.
RNA yield is poor	Confirm that the cell number is within the recommended range.
	Confirm that 80% sulfolane and cell lysate were combined at a 1:1 ratio prior to loading the RNA-binding spin cup.
	Incubate the spin cup for 2 full minutes after adding the Elution Buffer, then centrifuge for at least 5 minutes to collect the eluate.
	Warm the Elution Buffer to 60°C prior to application to the nano-spin cup matrix.
	Perform the elution twice or increase the volume of Elution Buffer. Any volume between 10 and 100 µl can be used.
Final RNA concentration is too low for use in subsequent applications	Concentrate the RNA under vacuum without heat.
DNA contamination	A highly viscous lysate will cause a large amount of genomic DNA to bind to the fiber matrix. Dilute the homogenate with additional Lysis Buffer or use a smaller number of cells.
	Ensure that the spin cup is centrifuged at maximum speed (≥12,000 × *g*) for 2 full minutes before DNase treatment.

APPENDIX I: ISOLATING RNA FROM CELLS HARVESTED BY LASER CAPTURE MICRODISSECTION

This protocol can be used with CapSure transfer film carriers [Arcturus Engineering, Inc. (*www.arctur.com*)] to isolate RNA from cells harvested by laser capture microdissection.

1. Add 0.7 µl of β-ME to 100 µl of Lysis Buffer for each sample.

2. For each sample, add 100 µl of the Lysis Buffer–β-ME mixture to an Eppendorf standard 0.5-ml microcentrifuge tube [Brinkmann Instruments, Inc. #22 36 430-8 (*www.brinkmann.com*)].

3. Snap a CapSure transfer film carrier containing laser-captured cells onto each microcentrifuge tube.

4. Invert and vortex tubes for 15–30 seconds to lyse the cells that were captured on the CapSure transfer film carrier.

5. Proceed with the protocol in *RNA Isolation from Cells*, beginning with the addition of 80% sulfolane at step 3.

APPENDIX II: PREVENTING SAMPLE CONTAMINATION

Preventing RNase Contamination

Ribonucleases are very stable enzymes that hydrolyze RNA. RNase A can be temporarily denatured under extreme conditions, but it readily renatures. RNase A can therefore survive autoclaving and other standard methods of protein inactivation. The following precautions can help prevent RNase contamination:

- **Wear gloves at all times** during the procedures and while handling materials and equipment, as RNases are present in the oils of the skin.
- Exercise care to ensure that all equipment and supplies (e.g., centrifuge tubes, etc.) are free from contaminating RNases. Avoid using equipment or workspaces that have been exposed to RNases. Use only sterile tubes and micropipet tips.
- Micropipettor bores can be a source of RNase contamination, since material accidentally drawn into the pipet or produced by gasket abrasion can fall into RNA solutions during pipetting. Clean micropipettors according to the manufacturer's recommendations. We recommend rinsing both the interior and exterior of the micropipet shaft with 70% ethanol or 70% methanol.

Sterilizing Labware

Disposable Plasticware

Disposable sterile plasticware is generally free of RNases. If disposable sterile plasticware is unavailable, components such as microcentrifuge tubes can be sterilized and treated with diethylpyrocarbonate (DEPC), which chemically modifies and inactivates enzymes, according to the following protocol:

Caution *DEPC is toxic and extremely reactive. Always use DEPC in a fume hood.* ***Read and follow the manufacturer's safety instructions.***

1. Add DEPC to deionized water to a final concentration of 0.1% (v/v) and mix thoroughly.
2. Place the plasticware to be treated into a separate autoclavable container. Carefully pour the DEPC-treated water into the container until the plasticware is submerged.

3. Leave the container and the beaker used to prepare DEPC-treated water in a fume hood overnight.

4. For disposal, pour the DEPC-treated water from the plasticware into another container with a lid. Autoclave the bottle of waste DEPC-treated water and the container with the plasticware for at least 30 minutes. Aluminum foil may be used to cover the container, but it should be handled with gloves and cut from an area untouched by ungloved hands.

Nondisposable Plasticware

Remove RNases from nondisposable plasticware with a chloroform rinse. Before using the plasticware, allow the chloroform to evaporate in a hood or rinse the plasticware with DEPC-treated water.

Electrophoresis Gel Boxes

To inactivate RNases on electrophoresis gel boxes, treat the gel boxes with 3% (v/v) hydrogen peroxide for 10–15 minutes and then rinse them with RNase-free water.

Glassware or Metal

To inactivate RNases on glassware or metal, bake the glassware or metal for a minimum of 8 hours at 180°C.

Treating Solutions with DEPC

Treat water and solutions (except those containing Tris base) with DEPC at 0.1% (v/v). During preparation, mix the 0.1% solution thoroughly, incubate the solution overnight at room temperature, and then autoclave it prior to use. If a solution contains Tris base, prepare the Tris solution with autoclaved DEPC-treated water.

Preventing Nucleic Acid Contamination

If the isolated RNA will be used for cDNA synthesis for cDNA library construction or PCR amplification, it is important to remove any residual nucleic acid from equipment that was used for previous nucleic acid isolations.

REFERENCES

1. Emmert-Buck, M. R., Bonner, R. F., Smith, P. D., Chuaqui, R. F., Zhuang, Z., *et al.* (1996) Science 274(5289): 998–1001.
2. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J. (1979) Biochemistry 18(24): 5294–9.
3. Vogelstein, B. and Gillespie, D. (1979) Proc Natl Acad Sci U S A 76(2): 615–9.

ENDNOTES

RiboGreen® is a registered trademark of Molecular Probes, Inc.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

An **Agilent Technologies** Division

Absolutely RNA Nanoprep Kit

QUICK-REFERENCE PROTOCOL

- Add 0.7 µl of β-ME to 100 µl of Lysis Buffer for each sample of ≤1 × 10^4 cells
- Add 100 µl of Lysis Buffer–β-ME mixture to each cell sample and vortex or pipet repeatedly until homogenized
- Add an equal volume of 80% sulfolane to the cell lysate and mix by vortexing for 5 seconds
- Transfer this mixture to a seated RNA-binding nano-spin cup; spin in a microcentrifuge at ≥12,000 × *g* for 60 seconds; **retain the spin cup** and discard the filtrate

Optional DNase Treatment

- Add 300 µl of 1× Low-Salt Wash Buffer and spin in a microcentrifuge at ≥12,000 × *g* for 60 seconds
- **Retain the spin cup** and discard the filtrate; spin in a microcentrifuge at ≥12,000 × *g* for 2 minutes to dry the filter
- Gently mix 2.5 µl of reconstituted RNase-Free DNase I with 12.5 µl of DNase Digestion Buffer
- Add the 15 µl of DNase solution directly onto the fiber matrix of the spin cup
- Incubate at 37°C for 15 minutes

- Add 300 µl of 1× High-Salt Wash Buffer and spin in a microcentrifuge at ≥12,000 × *g* for 60 seconds
- **Retain the spin cup** and discard the filtrate. Add 300 µl of 1× Low-Salt Wash Buffer and spin in a microcentrifuge at ≥12,000 × *g* for 60 seconds
- **Retain the spin cup** and discard the filtrate. Add 300 µl of 1× Low-Salt Wash Buffer and spin in a microcentrifuge at ≥12,000 × *g* for 60 seconds
- **Retain the spin cup** and discard the filtrate. Spin in a microcentrifuge at ≥12,000 × *g* for 3 minutes to dry the fiber matrix
- Transfer the spin cup to a fresh 2-ml collection tube
- Add 10 µl of Elution Buffer directly onto the fiber matrix and incubate for 2 minutes at room temperature; spin in a microcentrifuge at ≥12,000 × *g* for 5 minutes

The purified RNA is in the eluate in the collection tube. Transfer RNA to a capped microcentrifuge tube. Store at –80°C for the long term or at –20°C for the short term.