RNA Rapid Extraction Solution

Sample Recovery from Collection Cards for RNA Isolation

Part Number AM9775

Applied Biosystems

A. Background

Whatman® FTA® Cards

Whatman® FTA® Cards make use of a patented technology for collecting, transporting, and storing RNA and DNA in biological samples. "FTA Cards contain chemicals that lyse cells, denature proteins, and protect nucleic acids from nucleases, oxidation and UV damage."* Since captured nucleic acids are stabilized at room temperature, the cards facilitate sample collection in remote locations and simplify sample transport.

RNA Rapid Extraction Solution bridges the gap between sample collection and RNA isolation

Since many users of the Ambion® MagMAX™-96 Viral RNA Isolation Kit collect samples on FTA cards, we have developed the RNA Rapid Extraction Solution to bridge the gap between obtaining sample and isolating RNA. Disks punched from FTA Cards can be simply dropped into RNA Rapid Extraction Solution and agitated for 5 minutes to release the sample for RNA isolation—while protecting RNA from degradation. RNA can then be purified from the RNA Rapid Extraction Solution using the MagMAX-96 Viral RNA Isolation Kit. The high quality purified RNA is suitable for quantitative RT-PCR or other downstream RNA applications.

* Quoted from http://www.whatman.com/FTANucleicAcidCollectionStorageandPurification.aspx

B. Product Supplied and Storage Information

Part Number	Amount	
AM9775	10 mL	RNA Rapid Extraction Solution

Store RNA Rapid Extraction Solution at room temperature.

C. Required Materials Not Provided

- Whatman FTA Cards: there is an entire line of Whatman FTA Cards available for different applications; all of them are compatible for use with the RNA Rapid Extraction Solution. See www.whatman.com for a complete list.
- 2 mm puncher and cutting mat (e.g., Whatman Cat #WB100029)
- 96 well U-bottom microwell plate and lid (e.g., Phoenix Research Products Cat #MPU8117 and MPE 8019)
- Barnstead/Labline 96 well plate shaker model 4625 (e.g., VWR Cat #57019-600): we recommend this orbital shaker for its superior performance, however other vortexing shakers that accommodate 96 well plates may be used.

D. Related Products Available from Applied Biosystems

MagMAX™-96 Viral RNA Isolation Kit

P/N AM1836

The MagMAX™-96 Viral RNA Isolation Kit is designed for rapid high throughput purification of viral RNA in 96-well plates from biological fluids and cell-free samples such as serum, plasma, swabs, and cell culture media. As few as 20 copies of viral RNA can be detected from 50 µL of input sample.

E. Sample Compatibility and Collection

Sample compatibility

We have used the RNA Rapid Extraction Solution to obtain RNA from buccal swab, blood, and cultured cell samples, but any sample that is suitable for collection on FTA Cards is compatible with the solution.

1. Collect sample on FTA Card

Follow the procedure sent with the FTA Cards for specific instruction on collection of your sample type. The following instructions outline the procedures used at Ambion in developing the RNA Rapid Extraction Solution.

IMPORTANT

Always wear gloves when handling FTA Cards and avoid touching the pink sample area of the card to minimize cross-contamination.

Liquid suspension samples such as blood or serum or cultured cells

Drop 25–150 µL of sample directly onto the circle marked for sample on the FTA Card in a concentric circular pattern. Avoid puddling of liquid samples as this could overload the chemicals in the paper.

Buccal Samples

Press the sample collection swab or applicator onto the circular sample collection area of the FTA Card. Without lifting the swab from the card, rock it from side-to-side to squeeze the sample onto the card. Turn the swab over and repeat to collect sample from the other side.

2. Dry the FTA Card

Prop the samples up by folding the FTA Card as indicated in the card instructions. Allow the card to air dry at room temperature for at least one hour or until it is completely dry.

NOTE

To transport FTA Cards, we recommend following the manufacturer's recommendations and using desiccant (Whatman Cat #WB10-0003) and multi-barrier pouches (Whatman Cat #WB10-0010, WB10-0011).

F. Sample Extraction Procedure

IMPORTANT

Check the RNA Rapid Extraction Solution for precipitation. If precipitated material is visible (as may happen at low temperatures), heat the solution at 37°C for 5 min and invert the bottle a few times to bring the precipitate back into solution.

1. Punch disks from the card and place in a well of a 96-well plate

Punch one to three 2 mm disks from the sample spot on the FTA Card and place the disks into a well of a U-bottom 96-well plate.

2. Add 60–100 µL RNA Rapid Extraction Solution and shake 5 min

Add 60–100 µL of RNA Rapid Extraction Solution to each sample and shake for 5 min at moderate speed. On a Barnstead/Labline model 4625, this is setting 6. On other model shakers, use the maximum speed that does not risk spilling sample.

Alternatively, if a shaker is not available, use 100 µL of RNA Rapid Extraction Solution and let the samples soak for 20 min at room temperature.

3. Transfer 50 µL of the solution to a 96-well plate provided with the MagMAX-96 Viral RNA Isolation Kit

Transfer 50 µL of the RNA Rapid Extraction Solution into a U-bottom plate provided with MagMAX™-96 Viral RNA Isolation Kit.

If the samples were not shaken during the previous step, pipette up and down to mix well before transferring the 50 µL.

NOTE

If you used 60 µL of RNA Rapid Extraction Solution to recover sample from the FTA Card, you can transfer all of the solution to the MagMAX plate.

For RNA isolation, follow the procedure in the Protocol provided with the MagMAX™-96 Viral RNA Isolation Kit (available at www.ambion.com/catalog/CatNum.php?1836).

G. Troubleshooting

1. Samples precipitate when mixed with lysis/binding solution

If a precipitate forms when the MagMAX-96 Viral RNA Isolation Kit Lysis/Binding Solution is added to samples recovered in RNA Rapid Extraction Solution, it is due to evaporation of isopropanol from the Lysis/Binding Solution. This can be a problem when processing many plates at once. Avoid leaving the Lysis/Binding Solution in the tray for more than a few minutes because excessive exposure to air can cause evaporation of the isopropanol. Add the Lysis/Binding Solution to the samples right before processing.

If samples did precipitate when the Lysis/Binding Solution was added, before processing them further, add 30 µL of 100% isopropanol and pipet samples up and down until the precipitate is redissolved. This will not affect RNA recovery.

2. Cross contamination

FTA Card fibers can be retained on the card puncher causing cross contamination. To prevent this, consider the following suggestions:

- Use a sharp puncher so fewer fibers are carried over to the next sample.
- Clean the puncher with 70% ethanol and dry well using low-lint wipes between samples.
- Include a sample with blank FTA Card punches as a negative control for qRT-PCR or other downstream applications.

3. No RNA detected in qRT-PCR

More than one 2 mm punch from an FTA Card may be needed for samples with a low viral titer; up to six 2 mm FTA Card punches can be used per sample. Note that with 5–6 punches, you will need 100 µL RNA Rapid Extraction Solution.

If viral RNA is not detected even with 6 punches per sample, please refer to the troubleshooting suggestions in the MagMAX™-96 Viral Isolation Kit Protocol or contact our Technical Support Department.

H. Appendix

References

See Whatman's website for more information on using FTA Cards.

I. Quality Control

Functional Testing

RNA Rapid Extraction Solution is tested functionally using human blood spiked with an Armored RNA® transcript and spotted onto a Whatman FTA Card. The Armored RNA, hTBP mRNA and hTBP gDNA are then detected by qRT-PCR.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

J. Safety Information

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety goggles, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.

- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

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Literature Citation: When you are describing a procedure utilizing this product in a Materials and Methods Section for publication, we would appreciate that you refer to it as the RNA Rapid Extraction Solution Kit.

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