ChargeSwitch® Total RNA Cell Kits

For purification of total RNA from cells

Catalog nos. CS14010 and CS14010S

Version C

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User Manual

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Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Catalog no.	Number of Purifications
ChargeSwitch® Total RNA Cell Kit	CS14010	50
ChargeSwitch® Total RNA Cell Kit Sample Size	CS14010S	10

Shipping and Storage

All components of the ChargeSwitch® Total RNA Cell Kits, except the DNase I (shipped separately in dry ice), are shipped at room temperature.

Upon receipt, store components as follows:

- Store Proteinase K and Lysis Buffer (L16) at 4°C
- Store DNase I at -20°C
- Store the remaining components at room temperature

All components are guaranteed stable for 6 months when stored properly.

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Kit Contents and Storage, Continued

Contents

The components supplied in the ChargeSwitch® Total RNA Kits are listed below.

Note: Some reagents in the kit may be provided in excess of the amount needed.

Component	Amount	
	CS14010	**CS14010S**
ChargeSwitch® Magnetic Beads (25 mg/ml) in 1 mM sodium acetate, pH 4.5	5 ml	1 ml
Proteinase K (20 mg/ml) in 50 mM Tris-HCl, pH 8.5, 50% glycerol, and 5 mM $CaCl_2$	250 µl	250 µl
DNase I (1 U/µl) in 20 mM sodium acetate, pH 6.5, 5 mM $CaCl_2$, 0.1 mM PMSF, 50% glycerol	250 µl	250 µl
DNase I Buffer (40 mM Tris-HCl, pH 8.0, 10 mM $MgSO_4$, 3 mM $CaCl_2$)	12.5 ml	12.5 ml
Lysis Buffer (L16)	25 ml	25 ml
Binding Buffer (B9)	14 ml	14 ml
Wash Buffer (W13)	37.5 ml	37.5 ml
Wash Buffer (W14)	50 ml	50 ml
Elution Buffer (E7; 10 mM Tris-HCl, pH 9.0)	7.5 ml	7.5 ml

Product Qualification

Product Qualification

Each kit is functionally tested to ensure conformance with the most current approved product specifications.

Current specifications consist of tests for:

- Bead size, charge, and binding capacity
- Nucleic acid quality and quantity
- Buffer turbidity, volume, and absence of RNases and DNases
- Kit packaging and labeling accuracy

In addition, each kit component must be RNase-free and is lot-qualified for optimal performance. For individual lot test results and more information, visit www.invitrogen.com to download the Certificate of Analysis.

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® total RNA Cell Kit.

In addition, the table lists a selection of ChargeSwitch® Kits that are available for the purification of genomic DNA from various sample sources. For more information about these or other ChargeSwitch®, Kits, refer to our website at www.invitrogen.com or contact Technical Service (page 20).

Product	Amount	Catalog no.
MagnaRack™ Magnetic Rack	1 rack	CS15000
RNase *AWAY*®	250 ml	10328-011
0.1-2 Kb RNA Ladder	75 µg	15623-100
0.5-10 Kb RNA Ladder	75 µg	15623-200
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 ml	10977-015
UltraPure™ Dithiothreitol	5 g	15508-013
Quant-iT™ RNA Assay Kit	1000 assays	Q33140
ChargeSwitch® gDNA 20 µl Blood Kit	96 purifications	CS11010
ChargeSwitch® gDNA 100 µl Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 1 ml Serum Kit	50 purifications	CS11040
Phosphate Buffered Saline (PBS), 1X	500 ml	10010-023

Overview

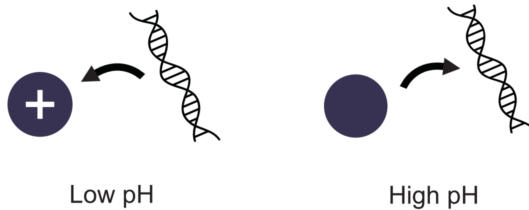
Introduction

The ChargeSwitch® Total RNA Cell Kits allow rapid and efficient purification of total RNA from cells. After preparing the lysates, you can purify the RNA in less than 15 minutes using the ChargeSwitch® Technology. For more information on the Charge Switch® Technology, see below.

The purified total RNA is suitable for use in any downstream application of choice (page 2).

The ChargeSwitch® Technology

The ChargeSwitch® Technology is a novel magnetic bead-based technology that provides a switchable surface charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification. In low pH conditions, the ChargeSwitch® beads have a positive charge that binds the negatively charged nucleic acid backbone (see figure below). Proteins and other contaminants are not bound and are simply washed away in aqueous wash buffers. To elute nucleic acids, the charge on the surface is neutralized by raising the pH to >8.5 using a low salt elution buffer (see figure below). Purified DNA or RNA elutes instantly into this elution buffer, and is ready for use in downstream applications.



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Advantages

Use of the ChargeSwitch® Total RNA Cell Kits provide the following advantages:

- Uses a magnetic bead-based technology to isolate high quality total RNA, including rRNA and the increasingly important small RNAs (smaller than 200 nucleotides)
- Eliminates the use of hazardous chemicals, common enzymatic inhibitors, centrifugation, or vacuum manifolds
- Rapid and efficient purification of total RNA from mammalian or bacterial cells in less than 15 minutes following sample preparation and lysis
- Minimal genomic DNA contamination of the purified RNA
- Reliable performance of the high-quality purified total RNA with improved performance in downstream applications compared to silica-based RNA purification

System Specifications

Starting Material:	Mammalian Cells (up to 1 x 10^6 cultured cells) Bacterial Cells (up to 1 ml culture, OD_{600} = 0.6 - 1.2)
Bead Binding Capacity:	~40 µg total RNA per mg
Bead Size:	< 1 µm
Bead Concentration:	25 mg/ml
Bead Storage Buffer:	1 mM sodium acetate, pH 4.5
Elution Volume:	~150 µl

RNA yields depend on the condition and type of cells.

Downstream Applications

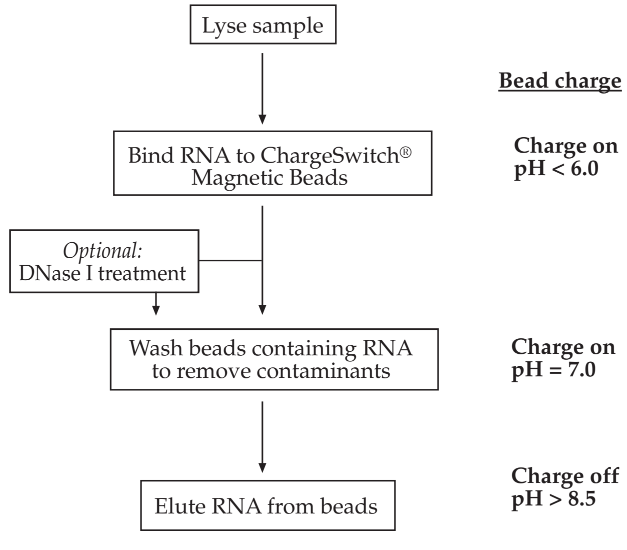
Total RNA isolated using the ChargeSwitch® Total RNA Cell Kits is suitable for:

Direct Use	Use after reverse transcription
Northern blotting	RT-PCR
Nuclease protection assays	Real time quantitative PCR (qPCR)
Reverse transcription	

Experimental Overview

Experimental Outline

The figure below illustrates the basic steps necessary to purify total RNA from cells using the ChargeSwitch® Total RNA Cell Kits.



General Information

To maximize the RNA yield, follow these guidelines for handling RNA when processing your samples:

- Use RNase *AWAY*® Reagent (page viii) to remove RNase contamination from surfaces.
- Maintain an RNAse-free working area while processing your samples.
- Use disposable, individually wrapped, sterile plastic ware.
- Use only sterile, new pipette tips and microcentrifuge tubes.
- Always wear latex gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin.
- Always use proper microbiological aseptic techniques when working with RNA.

MagnaRack™

You will need a magnetic rack (separator) for use with the ChargeSwitch® Total RNA Kits. We recommend using the MagnaRack™ to obtain the best results. Other magnetic separators may not provide similar magnetic strength or not be compatible with the volumes used in the protocol.

The MagnaRack™ available from Invitrogen (page viii) is a two-piece magnetic separation rack for use in protocols with magnetic beads. The MagnaRack™ consists of a magnetic base station and a removable tube rack. The tube rack can hold up to 24 microcentrifuge tubes. The tube rack fits onto the magnetic base station in two different positions associating the row of 12 neodinium magnets with a single row of 12 tubes for simple and easy 'on the magnet' and 'off the magnet' sample processing (see figure below). For more information, see www.invitrogen.com or contact Technical Service (page 20).

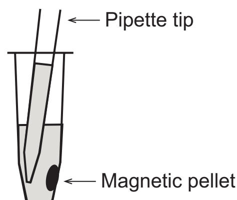
Continued on next page

General Information, Continued

Handling Magnetic Beads

Follow the recommendations below for best results:

- **Do not freeze the ChargeSwitch® Magnetic Beads** as freezing irreparably damages the property of the beads for nucleic acid purifications. Store the beads at room temperature.
- Always keep the beads in solution. **Do not allow the beads to dry** as this renders them non-functional.
- Before using the beads, resuspend thoroughly in the storage buffer by vortexing before removal from the storage tube.
- During the mixing and washing steps of the ChargeSwitch® Magnetic Beads, mix beads by pipetting up and down gently as directed in the protocol. You can also vortex at low speed to mix beads as directed in the protocol. To avoid any solution from collecting into the cap of the tube, always vortex at low speed.
- Always use an adjustable pipette set to a specific volume as directed in the protocol for mixing the contents by pipetting up and down gently to avoid forming bubbles.
- During all washing steps with beads, add buffer to the tube containing beads while the tube is still in the "on the magnet" position to prevent drying of beads. Subsequently, remove the tube from the magnet and resuspend the beads as described above.
- To aspirate the supernatant after bead washing, place the pipette tip away from the beads by angling the pipette such that the tip is pointed away from the pellet and carefully remove the supernatant without disturbing or removing any beads (see figure below).



- Discard beads after use. **Do not reuse the beads**.

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Elution Buffer

Elution Buffer

ChargeSwitch® Elution Buffer (E7; 10 mM Tris-HCl, pH 9.0) is provided in the kit for eluting total RNA from the ChargeSwitch® Magnetic beads. For best results, use Elution Buffer (E7) to elute the RNA. Alternatively, Tris Buffer, pH 8.5-9.0 in RNase-free water is acceptable. Note that if the pH of the buffer is <8.5, the RNA will not elute. **Do not use water for elution.**

Elution Buffer Volume

The protocol recommends eluting the RNA in 150 µl ChargeSwitch® Elution Buffer. You may vary the amount of ChargeSwitch® Elution Buffer to obtain total RNA in the desired final concentration.

For best results, always use a volume of ChargeSwitch® Elution Buffer (E7) that is equal or greater than the volume of ChargeSwitch® Magnetic Beads used in the protocol.

If the volume of ChargeSwitch® Elution Buffer is lower than the volume of beads used, RNA elution is incomplete. You may need to perform a second elution to recover all RNA.

Elution Buffer Temperature

Prewarming the elution buffer to 60-70°C increases the RNA yield.

Continued on next page

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® Total RNA Kits.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.

Sample Preparation

Introduction

Instructions for isolating total RNA from 1 x 10^6 mammalian cells or 1 ml overnight bacterial culture are described below. Read the entire instructions before starting the purification procedure.

Materials Needed

You will need the following items:

- Sample (up to 1 x 10^6 mammalian cells or 1 ml overnight bacterial culture, OD_{600} = 0.6 to 1.2)
- Sterile 1.5 ml microcentrifuge tubes
- Adjustable pipettes and aerosol barrier pipette tips
- 1X Phosphate Buffered Saline (PBS, page viii)
- **Fresh** 0.5 M DTT (RNase-free)
- Water bath or heat block set at 60°C
- Ice

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L16)
- Proteinase K

Preparing Reagents

Lysis Mix

For each sample, mix the following items in a microcentrifuge tube:

- 500 µl of ChargeSwitch® Lysis Buffer (L16)
- 5 µl fresh 0.5 M DTT
- 5 µl of Proteinase K

For multiple samples, prepare a master Lysis Mix by scaling up the volume of reagents accordingly.

Continued on next page

Preparing Mammalian Cell Lysates

1. Remove the growth medium from the cells.

   Note: Incomplete removal of the growth medium will inhibit the lysis and affect the efficiency of the RNA purification.

   Adherent cells (up to 1 x 10^6 cells): Completely remove the growth medium from the monolayer. Proceed with Step 2.

   Note: Depending on the cell type, you may use trypsin or a rubber policeman to remove all cells from the culture plate. Transfer the detached cells to a microcentrifuge tube and centrifuge briefly (depending on cell type) to pellet the cells. Completely remove the growth medium and proceed with Step 2.

   Suspension cells (up to 1 x 10^6 cells): Transfer the cells to a microcentrifuge tube and centrifuge briefly (depending on cell type) to pellet cells. Completely remove the growth medium. Proceed with Step 2.

2. Wash the cells with 1X PBS. Centrifuge detached cells or suspension cells by centrifugation. Completely remove PBS from cells.
3. Resuspend the cells in 500 µl of the prepared Lysis Mix, (previous page). Pipet up and down thoroughly (up to 15 times) until the cells are lysed and the lysate is no longer viscous.
4. Transfer the lysate into a 1.5 ml microcentrifuge tube.
5. Incubate at 60°C for 15 minutes.
6. After incubation, mix the lysate briefly by vortexing.
7. Cool the samples for 1 minute on ice.
8. Proceed to **Binding RNA**, page 12.

Continued on next page

Sample Preparation, Continued

Preparing Bacterial Lysates

For best results we recommend using log phase bacterial cells (OD_{600} = 0.6 to 1.2)

1. Harvest 1 ml overnight bacterial culture by centrifugation. Completely remove the growth medium.
2. Wash the cells with 1X PBS. Centrifuge the cells and completely remove the PBS from the cell pellet.

   Note: Incomplete removal of the growth medium will inhibit the lysis and affect the efficiency of the RNA purification.
3. Resuspend the cells in 500 µl Lysis Mix (page 8). Pipet up and down thoroughly (up to 15 times) until the pellet is broken up and the lysate is no longer viscous.
4. Incubate at 60°C for 15 minutes.
5. After incubation, vortex the lysate briefly to mix. Cool the samples for 1 minute on ice.
6. Proceed to **Binding RNA**, page 12.

Isolating Total RNA from Cells

Introduction

This procedure is designed for purifying total RNA from lysed mammalian cells (page 9) or lysed bacteria (page 10). The procedure includes an optional DNase I Treatment for purification of DNA-free total RNA.

To obtain high-quality total RNA, follow the guidelines recommended for handling RNA (page 4).

Materials Needed

You will need the following items:

- Sample lysate prepared as described in the **Sample Preparation** section (page 8).
- MagnaRack™ (page 4)
- Sterile 1.5 ml microcentrifuge tubes
- Adjustable pipettes and aerosol barrier pipette tips
- Water bath or heat block set at 60-70°C

Components Supplied with the Kit

- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Binding Buffer (B9)
- DNase I
- DNase I Buffer
- ChargeSwitch® Wash Buffer (W13)
- ChargeSwitch® Wash Buffer (W14)
- ChargeSwitch® Elution Buffer (E7)

Preparing Reagents

DNase I Digestion Mix

For optional DNase I digestion (page 13) mix for each sample the following items in a microcentrifuge tube:

- 5 µl DNase I enzyme
- 250 µl DNase I Buffer.

Mix well by pipetting. **Do not vortex.** For multiple samples, prepare a master DNase I Digestion Mix by scaling up the volume of reagents accordingly.

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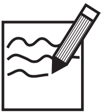
Binding RNA

Follow the procedure below to bind the RNA to the ChargeSwitch® Magnetic Beads.

1. **Thoroughly** vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in the storage buffer.
2. Add 100 µl of ChargeSwitch® Magnetic Beads to the lysate.
3. Add 200 µl Binding Buffer (B9) to the samples and mix by pipetting up and down gently 5 times using a 1 ml adjustable pipette tip set to 700 µl to mix the sample without forming bubbles.

   Important: Avoid forming bubbles by ensuring that the pipette tip is submerged during mixing, and by pipetting up and down gently.
4. Incubate at room temperature for 1 minute to allow the RNA to bind to the beads.
5. Place the sample on the MagnaRack™ until the beads have formed a tight pellet and the supernatant is clear.
6. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 5).
7. If DNA-free RNA is required proceed to **DNase I Treatment**, next page. If your downstream application tolerates the presence of genomic DNA, proceed to **Washing RNA**, page 14.



Note

The DNase I digestion can also be performed on the purified total RNA but this usually decreases the RNA yield.

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Isolating Total RNA from Cells, Continued

DNase I Treatment

Perform DNase I Treatment to prepare DNA-free RNA.

1. Remove the tube containing the pelleted magnetic beads from the magnet. There should be no supernatant in the tube.
2. Add 500 µl Wash Buffer (W14) to the tube and pipet up and down gently 5 times to resuspend the magnetic beads using a 1 ml pipette tip set to 700 µl to mix the sample without forming bubbles.
3. Place the sample on the magnet until the beads have formed a tight pellet and the supernatant is clear.
4. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet by angling the pipette such that the tip is pointed away from the pellet. Remove the tube from the magnet.
5. Add 250 µl of the prepared DNase I Mix (page 11). Resuspend the magnetic beads by pipeting up and down gently 5 times using a 1 ml pipette tip set to 200 µl to mix the sample without forming bubbles or vortex at low speed to avoid any solution from collecting in the cap of the tube.
6. Incubate at room temperature for 10 minutes.
7. Add 80 µl Binding Buffer (B9) to the sample and pipet up and down gently 5 times using a 1 ml pipette tip set to 200 µl to mix the sample without forming bubbles or vortex at low speed to resuspend the magnetic beads.
8. Incubate at room temperature for 1 minute.
9. Place the sample on the magnet until the beads have formed a tight pellet and the supernatant is clear.
10. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet by angling the pipette such that the tip is pointed away from the pellet.
11. Proceed to **Washing RNA**, next page.

Continued on next page

Washing RNA

Optional: If you wish to remove DNA from the sample, perform **DNase I Treatment** (previous page) before Washing RNA. If co-purification of genomic DNA does not affect downstream applications, proceed directly with the washing step.

1. Remove the tube containing the pelleted magnetic beads from the magnet (Step 6, page 12). There should be no supernatant in the tube.
2. Add 750 µl Wash Buffer (W13) to the tube. Resuspend the magnetic beads by pipeting up and down gently at least 5 times using a 1 ml pipette tip set to 700 µl to mix the sample without forming bubbles or vortex at low speed to avoid any solution from collecting in the tube cap.
3. Place the sample on the magnet until the beads have formed a tight pellet and the supernatant is clear.
4. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet by angling the pipette such that the tip is pointed away from the pellet. Remove the tube from the magnet.
5. Add 750 µl Wash Buffer (W14) or 500 µl if you performed DNase I treatment to the tube. Resuspend the magnetic beads by pipeting up and down gently 3 times to using a 1 ml adjustable pipette tip set to 700 µl to mix the sample without forming bubbles or vortex at low speed.
6. Place the sample on the magnet until the beads have formed a tight pellet and the supernatant is clear.
7. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet by angling the pipette such that the tip is pointed away from the pellet.
8. Proceed to **Eluting RNA**, next page.

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Isolating Total RNA from Cells, Continued

Eluting RNA

1. Remove the tube containing the pelleted magnetic beads from the magnet. There should be no supernatant in the tube
2. Add 150 µl Elution Buffer (E7) to the tube. Resuspend the magnetic beads by pipeting up and down gently 10 times using a pipette tip set to 120 µl to mix the sample or vortex at low speed.

   Note: See page 6 for more information on Elution Buffer volume. Pre-warming the Elution Buffer to 60-70°C may increase the RNA yield.
3. Incubate at room temperature for 5 minutes.

   Tip: For maximum yield, mix the suspension of beads after 2 minutes of incubation by pipetting up and down gently.
4. Place the sample on the magnet until the beads have formed a tight pellet and the supernatant is clear.
5. Without removing the tube from the magnet, carefully transfer the **supernatant containing the RNA** to a sterile microcentrifuge tube without disturbing the pellet by angling the pipette such that the tip is pointed away from the pellet. *The eluate volume is ~165 µl.*
6. Discard the used magnetic beads. **Do not** reuse the magnetic beads.
7. Store the purified total RNA at -80°C or use the RNA for the desired downstream application.

Analyzing RNA Yield and Quality

RNA Yield

Analyze the yield of purified total RNA by checking the UV absorbance at 260 nm or by using the Quant-iT™ RNA Assay Kits.

UV Absorbance

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.0. Mix well. Transfer to a cuvette (1-cm path length).

   Note: The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.
2. Determine the OD_{260} of the sample using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.0.
3. Calculate the amount of total RNA using the formula:

Total RNA (µg) = OD_{260} x 40 µg/(1 OD_{260} x 1 ml) x dilution factor x total sample volume (ml)

Quant-iT™ RNA Assay Kits

The Quant-iT™ RNA Assay Kit (page viii) provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a ready-to-use buffer. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers.

RNA Quality

Typically, RNA isolated using the ChargeSwitch® Total RNA Kits has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5) indicating that RNA is reasonably clean of proteins and other UV chromophores (heme, chlorophyl) that could interfere with downstream applications or negatively affect the stability of the stored RNA.

Agarose gel electrophoresis of purified RNA should show discreet 28S (5 kb) and 18S (1.9 kb) ribosomal RNA bands, and the 28S to 18S band ratio to be >1.5.

Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when purifying total RNA with the kit.

Problem	Cause	Solution
Low RNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add Proteinase K during lysis. • Increase the incubation time during lysis. • Ensure that the sample is fully homogenized before proceeding to 60°C incubation. • Depending on the type of adherent cells, it may be necessary to detach the cells using trypsin to ensure recovery of all cells. • Ensure that you completely remove the growth medium from the cells. • After removing the growth medium from the cells, wash the cells once in 1X PBS. Remove the PBS before adding the Lysis Mix to the cells.
	Quality of starting material	The yield and quality of RNA isolated depends on the cell type.
	Incorrect elution conditions	• After adding Elution Buffer (E7) to the sample, pipet up and down to resuspend the magnetic beads before incubation. • Increase the mixing steps during elution to 20-40 times or vortex at low speed for an additional 5-10 seconds. • Pre-heat the Elution Buffer at 60-70°C. • Always use Elution Buffer (E7) to elute the total RNA (page 6) for best results.

Continued on next page

Problem	Cause	Solution
Low RNA yield	RNA quantitation performed with water	Be sure the RNA quantitation using UV absorbance is performed with 10 mM Tris-HCl, pH 7.0 (page 16) to accurately measure the UV absorbance.
	Magnetic beads not functional	• Ensure that the magnetic beads are fully resuspended before use. • **Do not freeze the magnetic beads**. • Store the magnetic beads at room temperature. • **Do not reuse** the magnetic beads.
	Insufficient amount of ChargeSwitch® Magnetic Beads used for binding	Use the recommended amount of magnetic beads for binding. If RNA yields are lower, you may increase the amount of beads used. To compensate, also increase the amount of Binding Buffer (B9) by 10 µl for every additional 30 µl of beads used.
	Low RNA content	Various cells and tissues have different RNA content and the yield is dependent on the cell type. For low yielding samples, if concentration is too low, decrease the amount of beads used to enable a smaller amount of elution volume. To compensate, also reduce the amount of Binding Buffer (B9) by 10 µl for every additional 30 µl of beads used.
	Bubbles formed during mixing step	Ensure that the pipette tip is submerged in the solution during mixing.

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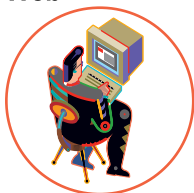
Troubleshooting, Continued

Problem	Cause	Solution
Eluate containing RNA is discolored	Magnetic pellet disturbed during elution	Place the sample on the MagnaRack™ ('on the magnet' position, page 4) until the beads form a tight pellet. Remove the eluate to a sterile microcentrifuge tube, taking care not to disturb the bead pellet.
DNA contamination	Improper DNase I digestion	• Store DNase I at -20°C immediately upon receipt. Do not store DNase I at room temperature. • Perform DNase I digestion as directed in the protocol.
RNA is degraded (gel electrophoresis analysis shows a smear)	Improper handling of RNA samples	Follow the recommendations on page 4 to prevent RNase contamination.

Technical Service

World Wide Web



Visit the Invitrogen Web site at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical service contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

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MSDS Requests

To request an MSDS, visit our Web site at **www.invitrogen.com.** On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Limited Warranty

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