invitrogen™
by life technologies™

iPrep™ PureLink™ Total RNA and TRIzol® Plus RNA Kits

For purification of total RNA from tissue, cells, and blood using the iPrep™ Purification Instrument

Catalog nos. IS-10006, IS-10007

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User Manual

Contents

Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of the iPrep™ PureLink™ Total RNA and iPrep™ TRIzol® Plus RNA Kits. For more details, refer to this manual.

Step	Procedure
Purification Protocol	1. Prepare samples as described on page 8 for iPrep™ TRIzol® Plus RNA Kit and page 12 for iPrep™ PureLink™ Total RNA Kit. 2. Open the iPrep™ Card Slot and insert the iPrep™ Card: Total RNA in the slot (arrow on the card is at the top and card label is facing your left side). 3. Turn **ON** the iPrep™ Instrument using the power switch on the left side of the instrument. The digital display shows the version for the iPrep™ instrument which changes in few seconds to display the Main menu. 4. Press **Start** to run a protocol. 5. Open the iPrep™ instrument door and remove iPrep™ Racks to set up the platform. 6. Remove the iPrep™ PureLink™ Total RNA Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube. 7. If you are performing DNase I digestion (available with iPrep™ PureLink™ Total RNA Kit only), insert one iPrep™ Sample and Elution Tube containing 1 µL DNase I in **position 11** (see page 3) **without caps** for each of the iPrep™ RNA Cartridge that is used. 8. Load the desired number of cartridges on the iPrep™ Cartridge Rack. Insert the loaded iPrep™ Cartridge rack on to the iPrep™ Platform.

Continued on next page

Experienced Users Procedure, Continued

Step	Procedure
Purification Protocol, Continued	9. Load the iPrep™ Tip and Tube Rack as follows: • Load the first row (labeled as **E**) with 1–13 elution tubes **without caps** • Keep the second row (labeled as **T1**) empty • Load the third row (labeled as **T2**) with iPrep™ Tips in the iPrep™ Tip Holders • Load the fourth row (labeled as **S**) with sample tubes **without caps** containing the lysate 10. Read the sample and elution tube barcodes, if needed. 11. Insert the iPrep™ Tip and Tube Rack on to the iPrep™ Platform. 12. Close the iPrep™ instrument door. Press **Enter** (↵) to continue. 13. Select the appropriate elution volume on the display. 14. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display. 15. At the end of the run, the instrument beeps briefly and the digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds. 16. Open the instrument door. 17. Remove and cap the elution tubes containing the purified nucleic acid. Aliquot and store the purified RNA at –80ºC. 18. Discard the used cartridges, tips, and tubes into biohazard waste. **Do not** reuse the cartridges. 19. To purify more samples using the same iPrep™ Card, load the racks with new cartridges, tips, tubes, and samples, and start the protocol as described. 20. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**. 21. Optional: Remove the iPrep™ Card and store card in the box.

Kit Contents and Storage

Types of Manuals

This manual is supplied with the following kits:

Product	Quantity	Cat. no.
iPrep™ PureLink™ Total RNA Kit	1 kit	IS-10006
iPrep™ TRIzol® Plus RNA Kit	1 kit	IS-10007

Kit Components

The table below shows the components supplied with iPrep™ PureLink™ Total RNA and TRIzol® Plus RNA Kits (iPrep™ RNA Kits).

Components	iPrep™ PureLink™ Total RNA Kit	iPrep™ TRIzol® Plus RNA Kit
iPrep™ Total RNA Box 1	√	√
iPrep™ PureLink™ Total RNA Box 2	√	
iPrep™ PureLink™ Total RNA Box 3	√	
TRIzol® Reagent		√

Shipping and Storage

The iPrep™ PureLink™ Total RNA and TRIzol® Plus RNA Kits (iPrep™ RNA Kits) are shipped as described below.

Upon receipt, store each component as described below.

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

Item	Shipping	Storage
iPrep™ Total RNA Box 1	Room temperature	Room temperature
iPrep™ PureLink™ Total RNA Box 2	Room temperature	Room temperature
iPrep™ PureLink™ Total RNA Box 3	Dry ice	–20ºC
TRIzol® Reagent	Room temperature	Room temperature

Continued on next page

Kit Contents and Storage, Continued

Box 1 Contents

The components supplied in iPrep™ RNA Kits, Box 1 are listed below.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
iPrep™ PureLink™ Total RNA Cartridge Kit	1 kit
iPrep™ Sample and Elution Tubes	3 × 52 tubes
iPrep™ Tips and Tip Holders	1 bag with 52 tips and holders

Box 2 Contents

The components supplied in iPrep™ PureLink™ Total RNA Kit, Box 2 are listed below.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
Lysis Buffer	32 mL
RBC Lysis Buffer	400 mL

Box 3 Contents

The iPrep™ PureLink™ Total RNA Kit, Box 3 includes 52 µL of DNase I (10 U/µL in 20 mM sodium acetate, pH 6.5, 5 mM calcium chloride, 50% glycerol, and 0.1 mM PMSF).

Sufficient DNase I is supplied to perform 52 purifications.

TRIzol® Reagent

The TRIzol® Reagent (100 mL) is supplied with the iPrep™ TRIzol® Plus RNA Kit only.

Sufficient TRIzol® Reagent is supplied to perform 52 purifications.

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Introduction

Product Overview

Introduction

The iPrep™ PureLink™ Total RNA and TRIzol® Plus RNA Kits (iPrep™ RNA Kits) allow rapid and automated extraction of total RNA from a variety of samples including tissue (plant and animal), cells (mammalian and bacterial cells), and fresh whole blood (iPrep™ TRIzol® Plus RNA Kit only).

Total RNA is extracted from samples using the Dynabeads® MyOne™ SILANE and iPrep™ Purification Instrument within 30–45 minutes without the use of centrifugation.

The purified total RNA is suitable for use in sensitive downstream applications including gene expression studies such as microarray analysis or real time quantitative RT-PCR (qRT-PCR).

iPrep™ Purification Instrument

The iPrep™ RNA Kits are designed for use with the iPrep™ Purification Instrument.

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe Unit capable of purifying nucleic acids from up to 13 samples (12 samples + 1 positive control) using a magnetic bead-based technology. See page 3 for details on the iPrep™ Purification Instrument.

System Overview

The iPrep™ RNA Kits combine the sensitivity and capacity of Dynabeads® MyOne™ SILANE with the speed and convenience of the iPrep™ Instrument to allow automated purification of high-quality RNA from up to 13 samples (12 samples + 1 positive control) within 30–45 minutes. The Dynabeads® MyOne™ SILANE are monodisperse magnetic beads (1 µm) with an optimized silica-like surface chemistry and a high specific surface area. Purification is achieved using a simple magnetic bead-based purification procedure, and avoids the use centrifuges or vacuum manifolds.

Samples are lysed manually using the Lysis Buffer, RBC Lysis Buffer, or TRIzol® Reagent (based on sample type). The lysate is mixed with Dynabeads® MyOne™ SILANE for subsequent RNA binding to the beads. The RNA-bound magnetic beads are separated from the lysate using magnetic separation. The beads are thoroughly washed with Wash Buffers to remove contaminants. The total RNA is then eluted in Elution Buffer.

Continued on next page

Product Overview, Continued

Advantages

The iPrep™ RNA Kits provide the following advantages:

- Uses a magnetic bead-based technology to isolate total RNA without the need for centrifugation or vacuum manifolds
- Rapid and automated purification of total RNA within 30-45 minutes from a wide range of samples including difficult tissue samples such as fatty and fibrous tissue
- Purifies fully representative RNA (includes high and low molecular weight RNA molecules)
- Pre-filled reagent cartridges provide easy set up and consistent results
- Minimal contamination with DNA
- Purified RNA demonstrates improved downstream performance in applications such as microarray analysis or real time quantitative RT-PCR (qRT-PCR)

System Specifications

Starting Material:	See page 6 for sample amount
Bead Size:	~1 µm
Bead Amount per Reaction:	2.4 mg
Number of Samples:	Up to 13
Elution Volume:	50 µL or 100 µL
RNA Yield*:	Varies (see page 21)

*The RNA yield depends on the sample type and quality.

iPrep™ Purification Instrument

Introduction

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe Unit capable of purifying nucleic acids from up to 12 samples and one positive control. Each iPrep™ Instrument consists of the Magnetic and Syringe Unit, and a platform. A pre-programmed iPrep™ Protocol Card controls the purification parameters such as buffer volumes, mixing steps, and incubation time.

For more details on the iPrep™ Purification Instrument, see the manual supplied with the instrument.

iPrep™ Reaction Cartridge

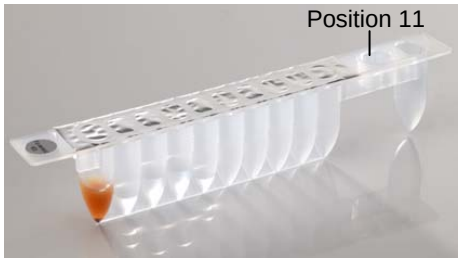
The iPrep™ Reaction Cartridges are supplied with iPrep™ Kits and are designed to fit onto the iPrep™ Cartridge Rack in only one orientation. Each cartridge is pre-filled with reagents required for the iPrep™ RNA protocol.

Each cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube). For the iPrep™ RNA Kits, positions 1–10 contain wells filled with reagents.

Cartridge Specifications:

Material:	Polypropylene cartridge sealed with laminated aluminum foil
Max Volume:	1000 µL/well
Dimension:	5.9 inches (l) × 1.2 inches (w) × 0.7 inches (d)

Note: The image below shows an example of an iPrep™ Reagent Cartridge and is not an image of an iPrep™ RNA Cartridge.



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iPrep™ Purification Instrument, Continued

iPrep™ Tips and Tip Holders

The iPrep™ Tips and Tip Holders are included with the iPrep™ Kits and are placed on the iPrep™ Tip and Tube Rack as described on page 18. While assembling the tips on the rack, insert the iPrep™ Tips into the iPrep™ Tip Holders using gloved hands. Always use the tips with the holders to prevent any contamination.

Tip Specifications:

Tip Material:	Polypropylene with filter barriers
Tip Holder Material:	Polypropylene
Volume:	5–1000 µL
Tip Dimension:	3.9 inches (l) × 0.43 inches (d)

iPrep™ Tip Holder

iPrep™ Tip

iPrep™ Tubes

Two sets of iPrep™ Tubes are required for the purification protocol. The iPrep™ Sample and Elution Tubes are included with each iPrep™ Kit and placed on the iPrep™ Tip and Tube Rack as described on page 18.

Tube Specifications:

Material:	Polypropylene
Capacity:	1.5 mL
Style:	Tubes with caps
Dimensions:	1.7 inches (l) × 0.4 inches (d)

Continued on next page

iPrep™ Purification Instrument, Continued

iPrep™ Card: Total RNA

To use the iPrep™ RNA Kits with the iPrep™ Purification Instrument, you need to purchase the iPrep™ Card: Total RNA (page 24).

The iPrep™ Card: Total RNA is pre-programmed with the purification protocol for RNA that directs the volume of reagents used and incubation time.

Always store the card in the box, protected from light.

To avoid damaging the card:

- Do not drop or bend the card
- Do not wipe or clean the card using volatile chemicals such as alcohol or equivalent
- Do not expose the card to water

iPrep™ Platform

The platform on the iPrep™ Instrument allows the placement of iPrep™ Tip and Tube Rack, and iPrep™ Cartridge Rack that are filled with plastic disposables and reagent cartridges required for the purification protocol.

Set up the platform as shown in the figure on page 18 for the iPrep™ RNA Kits.

Methods

General Information

User Supplied Materials

In addition to the reagents supplied with the kit, you also need the following materials and instrumentation:

- iPrep™ Purification Instrument (page 24)
- iPrep™ Card: Total RNA (page 24)
- Samples (see below)

Starting Material

The various sample types and amounts that can be processed using the system are listed in the table below:

Sample type	iPrep™ TRIzol® Plus RNA Kit	iPrep™ PureLink™ RNA Kit
Animal tissue	Up to 50 mg	Up to 10 mg
Mammalian cells	Up to 1×10^7 cells	Up to 1×10^6 cells
Plant tissue	100 mg	Not recommended
Fresh whole blood	Not recommended	Up to 1.0 mL
Bacterial cells	Up to 5×10^9	Up to 1×10^9

Guidelines for Handling RNA

Follow the guidelines below to prevent RNase contamination and to maximize the RNA yield:

- Use disposable, individually wrapped, sterile plastic ware
- Use only sterile, disposable RNase-free pipette tips and microcentrifuge tubes
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified material
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (page 24) to remove RNase contamination from work surfaces.

Continued on next page

Follow the recommendations below to obtain the best results:

- **Do not** freeze the beads as this irreparably damages them. Store the beads at room temperature.
- When using beads from the Reaction Cartridges, collect any solution from the foil by tapping the cartridge to deposit the solution at the bottom of the tube. **Do not** allow the beads to dry out as this renders them non-functional.
- Discard Reaction Cartridges, iPrep™ Tips, and iPrep™ Tip Holders after use. Do not reuse.

DNase I Digestion

The option to perform DNase I digestion is available with iPrep™ PureLink™ Total RNA Kits only. The DNase I digestion is performed during the purification protocol on the iPrep™ Instrument.

Place a tube containing 1 µL DNase I into the tube position of the cartridge (position 11, page 3). During the automated purification protocol, the DNase I Buffer included in the cartridge is added to the tube containing DNase I, mixed well, and DNase I is then added to the samples. Appropriate incubation time is included in the automated protocol to perform DNase I digestion.

Note: There is no need to perform the optional DNase I digestion step with the iPrep™ TRIzol® Plus RNA Kit as we have observed minimal DNA contamination using this kit.

Safety Information

Follow the safety guidelines below when using the iPrep™ RNA Kits.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles when handling whole blood samples.
- Dispose of blood samples as biohazardous waste.

Using the iPrep™ TRIzol® Plus Kit to Prepare Lysates

Introduction

Instructions for preparing lysates from mammalian cells and tissues, plant tissues, and bacterial cells using the buffers included with the iPrep™ TRIzol® Plus RNA Kit are described below.

See page 12 to prepare lysates using the iPrep™ PureLink™ Total RNA Kit.

To obtain high-quality RNA, follow the guidelines recommended on page 6.

Materials Needed

- Samples for RNA isolation (see page 6 for starting amounts)
- Chloroform
- Rotor stator homogenizer
- Liquid nitrogen, and mortar and pestle for plant tissues

Components Supplied with the Kit

- TRIzol® Reagent

Note

- Maintain frozen tissue at –80°C prior to lysis. Cool tubes in dry ice before placing frozen tissue in them. Thawing of frozen tissue prior to lysis may result in RNA degradation and loss of RNA yield.
- Fast and complete disruption of tissue during the lysis step is important to prevent RNA degradation.

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Using the iPrep™ TRIzol® Plus Kit to Prepare Lysates, Continued

Mammalian Tissue Lysate

Use the protocol below to prepare up to 50 mg of frozen or fresh tissue lysates.

1. Place up to 50 mg of freshly minced mammalian tissue or frozen tissue into a sterile tube placed on ice.
2. Add 1 mL TRIzol® Reagent supplied with the kit. Ensure the tissue is completely immersed in the buffer.
3. Homogenize the tissue for a minimum of 1 minute using a hand held rotor stator tissue homogenizer.
4. Incubate the samples at room temperature for 5 minutes.
5. Add 0.2 mL chloroform and shake the tube vigorously by hand for 15 seconds. Avoid vortexing the sample.
6. Incubate at room temperature for 2–3 minutes.
7. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.

   After centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. The volume of aqueous phase is ~600 µL.
8. Transfer ~400–500 µL of the colorless, upper phase containing RNA to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Mammalian Cells Lysate

Procedure to prepare lysate from mammalian cells is described below.

1. For adherent cells (up to 1×10^7 cells), remove the growth medium from the culture plate.

   For suspension cells (up to 1×10^7 cells), harvest the cells and centrifuge the cells at 250 × *g* for 5 minutes to pellet cells. Remove the growth medium.
2. Resuspend the pelleted cells using 1 mL TRIzol® Reagent.
3. Homogenize the sample for a minimum of 1 minute using a hand held rotor stator tissue homogenizer.
4. Incubate at room temperature for 5 minutes.
5. Add 0.2 mL chloroform and shake the tube vigorously by hand for 15 seconds. Avoid vortexing the sample.

Continued on next page

Using the iPrep™ TRIzol® Plus Kit to Prepare Lysates, Continued

Mammalian Cells Lysate, Continued

6. Incubate at room temperature for 2–3 minutes.
7. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.
8. After centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. The volume of aqueous phase is ~600 µL.
9. Transfer ~400–500 µL of the colorless, upper phase containing RNA to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Plant Lysate

Procedure to prepare plant tissue lysate is described below.

1. Place up to 100 mg of plant tissue in a pre-chilled mortar.
2. Immediately add liquid nitrogen and grind the tissue to a fine powder. Do not allow the liquid nitrogen to completely evaporate.
3. Transfer the ground powder with remaining liquid nitrogen to a fresh RNase-free tube.
4. Place the tube on dry ice and allow the liquid nitrogen to evaporate.
5. Add 1 mL TRIzol® Reagent and homogenize the sample for 2 minutes using a hand held rotor stator homogenizer.
6. Incubate at room temperature for 5 minutes.
7. Add 0.2 mL chloroform and shake the tube vigorously by hand for 15 seconds. Avoid vortexing the sample.
8. Incubate at room temperature for 2–3 minutes.
9. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.
10. After centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. The volume of aqueous phase is ~600 µL.
11. Transfer ~400–500 µL of the colorless, upper phase containing RNA to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

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Using the iPrep™ TRIzol® Plus Kit to Prepare Lysates, Continued

E. coli Lysate

Procedure to prepare *E. coli* cell lysate is described below.

1. Harvest log-phase bacteria (1 mL of overnight culture with OD_{600} up to 2.0 or up to 5×10^9 cells) by centrifugation at 500 × *g* for 5 minutes at 4°C.

   If you are using a frozen cell pellet, proceed directly to Step 2.
2. Resuspend the cell pellet in 1 mL TRIzol® Reagent and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds.
3. Add 0.2 mL chloroform and shake the tube vigorously by hand for 15 seconds. Avoid vortexing the sample.
4. Incubate at room temperature for 2–3 minutes.
5. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.
6. After centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. The volume of aqueous phase is ~600 µL.
7. Transfer ~400–500 µL of the colorless, upper phase containing RNA to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Using the iPrep™ PureLink™ Kit to Prepare Lysates

Introduction

Instructions for preparing lysates from mammalian cells and tissues, whole blood, and bacterial cells using the buffers included with the iPrep™ PureLink™ Total RNA Kit are described below.

See page 8 to prepare lysates using the iPrep™ TRIzol® Plus RNA Kit.

To obtain high-quality RNA, follow the guidelines recommended on page 6.

Materials Needed

- Samples for RNA isolation (see page 6 for starting amounts)
- β-mercaptoethanol
- Rotor stator homogenizer
- 10% SDS and 10 mg/mL lysozyme solution in TE for bacterial samples
- Optional: Homogenizer (page 24) for tissue samples

Components Supplied with the Kit

- Lysis Buffer
- RBC Lysis Buffer

Note

- Maintain frozen tissue at –80°C prior to lysis. Cool tubes in dry ice before placing frozen tissue in them. Thawing of frozen tissue prior to lysis may result in RNA degradation and loss of RNA yield.
- For samples that are difficult to lyse, use TRIzol® Reagent as described on page 8.
- Fast and complete disruption of tissue during the lysis step is important to prevent RNA degradation.

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Using the iPrep™ PureLink™ Kit to Prepare Lysates, Continued

Mammalian Tissue Lysate

Use the protocol below to prepare up to 10 mg of frozen or fresh tissue lysates.

1. Place up to 10 mg of freshly minced mammalian tissue or frozen tissue into a sterile tube placed on ice.
2. Add 600 µL Lysis Buffer supplied with the kit. Ensure the tissue is completely immersed in the buffer.
3. Homogenize the tissue for a minimum of 1 minute using a hand held rotor stator tissue homogenizer.
4. Centrifuge the lysate at 12,000 × *g* for 5 minutes at room temperature to remove any particulate material

   Note: If the lysate is viscous or contains cell debris, clarify the lysate using the Homogenizer available from Invitrogen (page 24).
5. Transfer the supernatant to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Mammalian Cells Lysate

Procedure to prepare lysate from mammalian cells is described below.

1. For adherent cells (up to 1×10^6 cells), remove the growth medium from the culture plate.

   For suspension cells (up to 1×10^6 cells), harvest the cells and centrifuge the cells at 250 × *g* for 5 minutes to pellet cells. Remove the growth medium.
2. Resuspend cells in 600 µL Lysis Buffer supplied with the kit. Mix well by vortexing or pipetting up and down until the cells appear lysed.
3. Homogenize the sample for a minimum of 2 minutes using a hand held rotor stator tissue homogenizer.
4. Transfer the cell lysate to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Continued on next page

Using the iPrep™ PureLink™ Kit to Prepare Lysates, Continued

Whole Blood Sample Preparation

Procedure to prepare whole blood sample is described below. To isolate RNA from blood, you need to first isolate the white blood cell (WBC) fraction and then lyse the WBC to prepare whole blood lysates.

1. To up to 1 mL of **fresh,** whole blood sample, add 5 volumes of RBC Lysis Buffer supplied with the kit.

   For example: To 1 mL of **fresh**, whole blood, add 5 mL RBC Lysis Buffer.
2. Incubate samples on ice for 10 minutes with intermittent vortexing every 3 minutes.
3. Centrifuge samples at 400 × *g* for 10 minutes at 4ºC. Remove and discard the supernatant.
4. Resuspend the pellet in 2 volumes of RBC Lysis Buffer. Mix well by vortexing.

   For example: Resuspend the pellet in 2 mL (for 1 mL starting volume of blood used) RBC Lysis Buffer.
5. Centrifuge samples at 400 × *g* for 10 minutes at 4ºC. Remove and discard the supernatant.
6. Resuspend the resulting WBC pellet in 600 µL Lysis Buffer.
7. Homogenize the cells using a rotor-stator homogenizer at maximum speed for at least 45 seconds.
8. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes at room temperature.
9. Transfer the lysate to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Continued on next page

Using the iPrep™ PureLink™ Kit to Prepare Lysates, Continued

E. coli Lysate

Procedure to prepare *E. coli* cell lysate is described below.

1. Prepare 100 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing 1 mg lysozyme enzyme. Store on ice until use. You will need 100 µL lysozyme solution/sample.
2. Using the Lysis Buffer included with the kit, prepare 500 µL Lysis Buffer containing 10 µL β-mercaptoethanol per sample.
3. Harvest log phase bacteria (0.5 mL of overnight culture with OD_{600} up to 1.0 or up to 1×10^9 cells) by centrifugation at 500 × *g* for 5 minutes at 4°C.

   If you are using a frozen cell pellet, proceed to Step 4.
4. Resuspend the cell pellet in 100 µL TE buffer containing 1 mg lysozyme from Step 1.
5. Add 0.5 µL 10% SDS to the lysate and mix well by vortexing. Incubate at room temperature for 5 minutes.
6. Add 500 µL Lysis Buffer containing β-mercaptoethanol (Step 2) to the lysate.
7. Homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds.
8. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes at room temperature.
9. Transfer the lysate to an iPrep™ Sample Tube and proceed to **Isolating Total RNA** using the iPrep™ Instrument (page 17).

Isolating Total RNA

Introduction

Instructions to isolate total RNA using the iPrep™ RNA Kits with the iPrep™ Purification Instrument are described below.

Materials Needed

- Lysate (prepared as described on pages 8–12)
- iPrep™ Purification Instrument (page 24)
- iPrep™ Card: Total RNA (page 24)

Components Supplied with the Kit

- iPrep™ PureLink™ RNA Cartridge Kit
- iPrep™ Sample and Elution Tubes
- iPrep™ Tips
- iPrep™ Tip Holders
- DNase I (optional)

Before Starting

Perform the following before starting:

- Prepare lysates as described on pages 8–12
- Ensure that you have the iPrep™ Card: Total RNA (page 24) to run the protocol
- Make sure the iPrep™ Purification Instrument is unpacked and installed

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Isolating Total RNA, Continued

Purification Protocol

Purify total RNA using the iPrep™ Purification Instrument as described below.

For details on using the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

Insert the iPrep™ Card: Total RNA (available separately from Invitrogen, page 24) prior to turning on the instrument.

1. Ensure the power switch on the iPrep™ Instrument is on the **OFF** position.
2. Open the iPrep™ Card Slot and insert the iPrep™ Card: Total RNA into the slot in the correct orientation (arrow on the card is at the top and card label is facing your left side).
3. Using the power switch located on the left side of the instrument, turn **ON** the instrument.

   If the card is fully inserted in the correct orientation, all axes return to their original positions automatically. The digital display shows the version for the iPrep™ which changes in few seconds to display the Main menu.
4. Press **Start** to run a protocol.
5. Open the iPrep™ instrument door. Remove the iPrep™ Cartridge Rack, and iPrep™ Tip and Tube Rack to set up the platform.
6. Remove the desired number of iPrep™ PureLink™ Total RNA Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.

   Note: You can load 1–13 cartridges on the rack depending on the number of samples that you wish to process. If you are loading less than 13 cartridges, ensure that the remaining plastic ware (tips and tubes) are also loaded in the same order as the cartridges.

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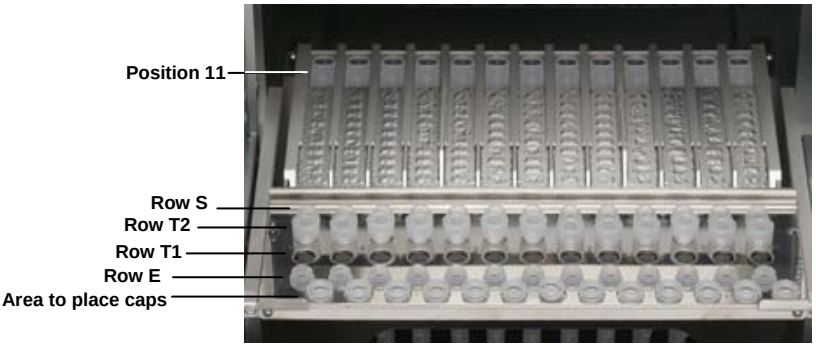
Isolating Total RNA, Continued

Purification Protocol, Continued

Procedure continued from previous page

7. If you are performing DNase I digestion (available with **iPrep™ PureLink™ Total RNA Kit only**) for your samples, insert one iPrep™ Sample and Elution Tube containing DNase I **without caps in position 11** for each of the iPrep™ RNA Cartridge that is used.
8. Load the cartridges on the iPrep™ Cartridge Rack and insert the loaded rack on the iPrep™ platform.
9. Load the iPrep™ Tip and Tube Rack as follows (see figure below):
   - Load the first row (labeled as **E**) with 1–13 elution tubes **without caps** (you may place the caps on the rack as shown in the figure below)
   - Keep the second row (labeled as **T1**) empty
   - Load the third row (labeled as **T2**) with iPrep™ Tips in the iPrep™ Tip Holders
   - Load the fourth row (labeled as **S**) with sample tubes **without caps** containing samples



10. Read the sample and elution tube barcode, if needed.
11. Insert the iPrep Tip and Tube rack on the iPrep™ platform as shown above.
12. Close the iPrep™ instrument door.

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Isolating Total RNA, Continued

Purification Protocol, Continued

Procedure continued from previous page

13. Press **Enter** (↵)to continue.
14. Select the appropriate protocol followed by elution volume on the display, when prompted.
15. Ensure that you have loaded the cartridges, tubes, and tips in the appropriate positions, and elution tubes **do not have any caps. Make sure you have loaded a tube containing the sample in the heated tube position (position 11) of the cartridge.**
16. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display.

    Important: Do not open the door once the protocol has begun.

    To pause the protocol, press the **Stop** key. To resume the protocol after a pause, press the **Start** key. To cancel/stop the protocol, press the **Stop** key twice. For details, see the iPrep™ Instrument manual.
17. At the end of the run, the instrument beeps briefly and the digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds.
18. Open the instrument door. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified RNA as described below.
19. Discard the used cartridges, tips, and sample tubes into biohazard waste. **Do not reuse the cartridges.**
20. To purify more samples using the same iPrep™ Card, load the racks with new cartridges, tips, and samples, and start the protocol as described above.
21. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**.

Note: Remove the iPrep™ Card and store the card in the box, protected from light, if you are planning to use the instrument with a different iPrep™ Card. If the instrument is repeatedly used with the same iPrep™ Card, you can keep the card in the instrument.

Storing RNA

- Store the RNA on ice, if you will use the RNA within a few hours for the desired downstream application.
- Aliquot purified RNA and store at –80°C. Avoid repeated freezing and thawing.

RNA Quantitation and Analysis

RNA Yield

Total RNA is easily quantitated using the Quant-iT™ RNA Kits or UV absorbance at 260 nm.

Quant-iT™ RNA Kits

The Quant-iT™ RNA Kit (page 24) 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 kits contain a state-of-the-art quantitation reagent and pre-diluted standards for standard curve. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers/fluorometers.

UV Absorbance

To determine the quantity by UV absorbance:

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.5. 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 solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.
3. Calculate the amount of total RNA using the following formula:

   Total RNA (µg) = $A_{260} \times 40\ \mu g/(A_{260} \times 1\ mL)] \times$ dilution factor × total sample volume (mL)

Analyzing RNA Quality

Typically, total RNA isolated using the iPrep™ RNA Kits has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5). An $OD_{260/280}$ of >1.8 indicates that RNA is reasonably clean of proteins and other UV chromophores that could either interfere with downstream applications or negatively affect the stability of the stored RNA. RNA quality may also be assessed using a bioanalyzer.

Agarose gel electrophoresis of RNA isolated using the iPrep™ RNA Kits show the 28S to 18S band ratio to be >1.5. RNA is judged to be intact if discreet 28S and 18S ribosomal RNA bands are observed.

Expected Results

Expected Yield and Purity

Examples of expected yield and purity are given as mean values, with the range of values in parentheses.

Total RNA was purified using the iPrep™ RNA Kits and the iPrep™ Purification Instrument using different samples. RNA was eluted in 100 µL elution buffer; yield was determined using the Quant-iT™ RNA Assay Kit. The UV absorbance ratios were measured using a NanoDrop® ND-1000 spectrophotometer.

Sample	iPrep™ TRIzol® Plus RNA Kit		iPrep™ PureLink™ Total RNA Kit	
	Yield (µg)	A_{260}/A_{280}	Yield (µg)	A_{260}/A_{280}
293 cells ($1 \times 10^7/1 \times 10^6$)	52.2 (50.1–55.5)	2.0	10.9 (10.1–11.6)	2.0
Liver (10 mg)	44.2 (43.3–46.3)	2.1	40.8 (35.9–44.5)	2.1
Heart (10 mg)	4.8 (3.7–6.4)	1.9	1.9 (1.1–2.3)	2.0
Kidney (10 mg)	9.2 (7.2–12.3)	1.9	7.0 (5.8–8.7)	2.1
E. coli (0.5 mL culture, OD_{600} = 1)	80.3 (79.0–81.7)	1.9	64.8 (58.0–68.5)	2.0
Tomato leaf (100 mg)	23.9 (23.1–25.4)	2.0	kit not recommended for this sample type	

Troubleshooting

Introduction

Refer to the table below to troubleshoot problems with the kit. To troubleshoot problems with the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

Observation	Cause	Solution
Low RNA yield	Incomplete lysis and homogenization	• Perform lysis and homogenization as recommended for each sample type using the appropriate lysis buffer as described on pages 8–12. • Decrease the amount of starting material. • Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in buffer to achieve optimal lysis.
	Poor quality of starting material	• The yield and quality of RNA isolated depends on the type and age of the starting material. • Be sure to use fresh sample and process immediately after collection, or freeze the sample at –80ºC or in liquid nitrogen immediately after harvesting.
	Insufficient amount of Dynabeads® MyOne™ SILANE added	During shipping, some Dynabeads® MyOne™ SILANE solution may adhere to the sealing foil of the cartridge. To collect any bead solution from the foil, tap the cartridge to deposit the bead solution at the bottom of the tube.
	Used less than the recommended sample amount	See page 6 for recommended starting amount for various sample types depending on the type of kit you are using.
No RNA recovered	Magnetic beads stored or handled improperly	• Store cartridge containing the beads at room temperature. Do not freeze the cartridge as the beads may be irreparably damaged. • Make sure the beads are in solution at all times and do not dry. Dried beads are non-functional.
	Too much starting material clogs tips	Decrease the amount of starting material use. See page 6 for recommended starting amount. Make sure the lysates is clear and does not contain particulate material.

Continued on next page

Troubleshooting, Continued

Observation	Cause	Solution
RNA eluate is discolored	Magnetic beads present in the eluate	Remove any magnetic beads using a magnetic separator (MagnaRack™ is available from Invitrogen, see page 24) or centrifuge the sample in a microcentrifuge for 1 minute at maximum speed.
RNA is degraded	RNA contaminated with RNase	• Use RNase-free pipette tips with aerosol barriers. • Change gloves frequently. • Swipe automatic pipettes with RNase *AWAY*™ solution.
	Improper handling of sample from harvest until lysis	• If not processed immediately, quick-freeze tissue immediately after harvesting and store at –80ºC or in liquid nitrogen. • Frozen samples must remain frozen until Lysis Buffer or TRIzol® Reagent is added. • Perform the lysis quickly after adding Lysis Buffer or TRIzol® Reagent.
DNA contamination	DNase I not added during the purification protocol	Be sure to add a tube containing 1 µL DNase I supplied with iPrep™ PureLink™ Total RNA Kit in position 11 of the iPrep™ RNA Cartridge to perform DNase I digestion during the automated purification protocol.

Appendix

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the iPrep™ RNA Kits.

For more information, visit www.invitrogen.com or contact Technical Support (page 25).

Product	Amount	Cat. no.
iPrep™ Purification Instrument	1 unit	IS-10000
iPrep™ Card: Total RNA	1 card	IS-10014
iPrep™ Card: gDNA Blood	1 card	IS-10012
iPrep™ Card: gDNA Tissue	1 card	IS-10013
iPrep™ Card: gDNA Forensic (includes buccal protocol)	1 card	IS-10011
iPrep™ PureLink™ gDNA Blood Kit	1 kit	IS-10005
iPrep™ ChargeSwitch® Forensic Kit	1 kit (52 purifications)	IS-10002
iPrep™ ChargeSwitch® Buccal Cell Kit	1 kit (52 purifications)	IS-10003
iPrep™ ChargeSwitch® gDNA Tissue Kit	1 kit (52 purifications)	IS-10004
iPrep™ Tip and Tube Rack	1 rack	IS-10101
iPrep™ Cartridge Rack	1 rack	IS-10102
Quant-iT™ RNA Assay Kit, 1000 assays *5–100 ng*	1 kit	Q33140
Qubit® Fluorometer	1 each	Q32857
MagnaRack™ Magnetic Separator	1 rack	CS15000
Homogenizer	1 pack of 50	12183-026
RNase *AWAY*™ Reagent	250 mL	10328-011

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless, pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats. In addition, a large variety of DNA ladders is available from Invitrogen for sizing DNA. For more information about these products, see www.invitrogen.com or call Technical Support (page 25).

Technical Support

World Wide Web

Visit the Invitrogen website at **www.invitrogen.com** for:

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

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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Continued on next page

Technical Support, Continued

SDS Requests

SDSs (Safety Data Sheets) are available on our website at www.invitrogen.com/sds.

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