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QIAsprint® RNA Tissue/Cells Application Guide

For automated purification of total RNA including small RNAs

Sample to Insight

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Required Kits and Components

For isolation of RNA from cells and tissue samples, the following kits and components are required:

Required kit/component name	Cat. no.
QIAsprint RNA Tissue/Cells PrepSet (384)	586549
QIAsprint Essential Kit B (384)	586009
QIAsprint Prep Cover (8)	582103
QIAsprint Prep Plate Insert (32)	582226
QIAsprint Elution Plate (10)	583204

Optional kit/component name	Cat. no.
QIAsprint DNA Removal Set (384)	586409

Kit Contents

The QIAsprint Connect offers the possibility of a modular kit concept, and the following combination is recommended for purification of total RNA including miRNA from cells and tissue samples.

QIAsprint RNA Tissue/Cells PrepSet **Catalog no.** **Number of preps**	**(384)** **586549** **384**
Buffer RTL*	3 × 45 mL
Proteinase K	10 mL
RNase-free water	30 mL

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 9 for Safety Information.

QIAsprint Essential Kit B **Catalog no.** **Number of preps**	**(384)** **586009** **384**
Buffer RPE (concentrate)	3 × 55 mL
RNase-free water	30 mL
RNase-free water	50 mL
MagG Bead Suspension	12 mL

QIAsprint DNA Removal Set Catalog no. Number of preps	(384) 586409 384
DNase I, RNase-Free (lyophilized)	8 × 1500 Kunitz units*
RNase-free water	10 mL
Buffer RDW	30 mL

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A260 of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. 33, 349 and 363).

Shipping and Storage

The QIAsprint RNA Tissue/Cells PrepSet and QIAsprint Essential Kit B are shipped at ambient temperature.

All buffers and reagents should be stored at room temperature (15–25°C) until the expiration date printed on the box label.

QIAsprint DNA Removal Set (cat. no. 586409) is required for extraction of gDNA-free RNA. Store all components of this set at room temperature (15–25°C) except DNase I, which must be stored at 4°C.

Intended Use

The QIAsprint RNA Tissue/Cells PrepSet is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and components.

CAUTION DO NOT add bleach or acidic solutions directly to the sample preparation waste.



Buffer RLT contains chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsprint RNA Tissue/Cells PrepSet is tested against predetermined specifications to ensure consistent product quality.

Introduction

The application guide describes processing of the QIAsprint RNA Tissue/Cells PrepSet and the QIAsprint Essential Kit B with the QIAsprint Connect instrument. For a detailed description of the QIAsprint Connect instrument, refer to the respective user manual (**www.qiagen.com/HB- 3708**) and quick- start guide (**www.qiagen.com/HB- 3709**). The QIAsprint RNA Tissue/Cells application enables automated high-throughput purification of RNA and miRNA from cells and tissue samples using the QIAsprint Connect instrument.

The QIAsprint RNA Tissue/Cells PrepSet and the QIAsprint Essential Kit B are for purification of total RNA including miRNA from cells and tissue samples. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready to use for highly sensitive detection in downstream assays, such as amplification, or other enzymatic reactions. The QIAsprint Connect can process up to 96 samples per run, and subsequent runs allow handling of up to 192 samples in a single session.

Automated purification on QIAsprint Connect

Nucleic acid purification is automated on the QIAsprint Connect platform. The advanced technology integrated into QIAsprint Connect ensures efficient purification of nucleic acids for a wide range of applications while offering exceptional flexibility to allow customers to customize their purification protocols according to specific requirements.

QIAsprint Connect provides comprehensive protocols and purification guides for plasmid DNA, gDNA, RNA, and viral/bacterial nucleic acids.



Figure 1. The QIAsprint Connect instrument.

Principle and procedure

The QIAsprint RNA Tissue/Cells application combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples. The purification procedure comprises four steps: lyse, bind, wash, and elute.

Lysis with Proteinase K

Lysis is performed in the presence of Proteinase K and lysis buffer, which together ensure digestion of proteins and inactivation of RNases.

Binding to magnetic particles

The QIAsprint RNA Tissue/Cells application provides a fully automated protocol for the purification of RNA from cells and tissue samples. Before loading samples onto the QIAsprint Connect instrument, the procedure begins with a manual lysis step using Buffer RLT, which protects RNA molecules. This step is followed by Proteinase K digestion under optimized conditions to ensure complete lysis of even difficult-to-lyse tissues and efficient release of RNA. Immediately before starting the run, isopropanol and magnetic beads are added to the sample to enable optimal adsorption of RNA to the silica surface.

Washing of bound nucleic acids

While the nucleic acids remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps To purify RNA only, an optional DNase digestion using the QIAsprint DNA Removal Set must be performed.

Elution of purified nucleic acids

In a single step, highly purified RNA is eluted in RNase-free water. The purified nucleic acid can be either used immediately in downstream applications or stored for future use.

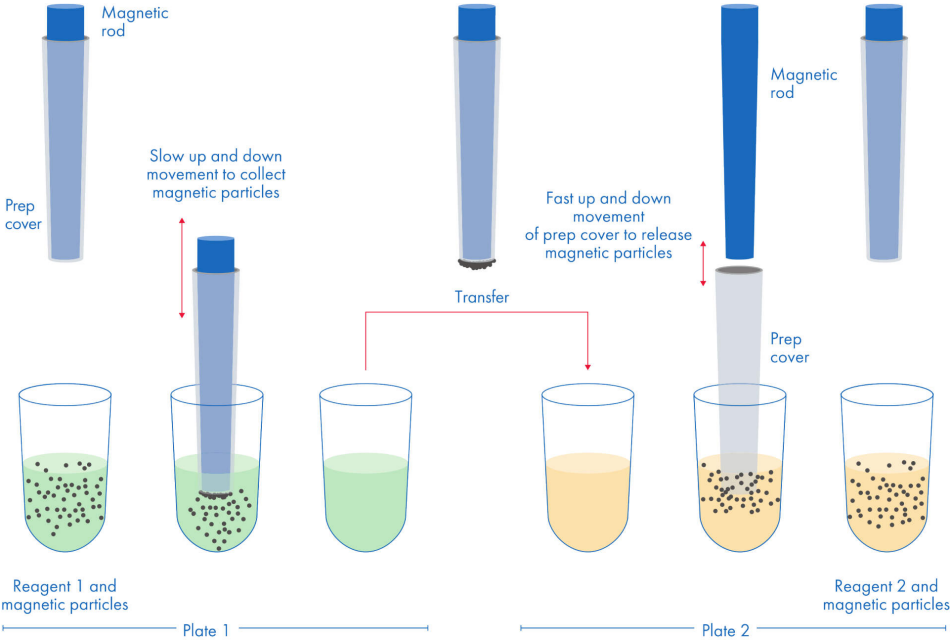


Figure 2. Handling of magnetic particles by the QIAsprint Connect instrument.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

Required materials not supplied

- Pipettes and sterile, RNase-free pipette tips
- Disposable gloves
- 96–100% Ethanol*
- Isopropanol
- **Optional**: QIAsprint Frame Shield (100) (cat. no. 580010)

  Note: If desired, use QIAsprint Frame Shield to minimize adhesions of beads to the QIAsprint Prep Cover Frame (cat. no. 582111). For this, place QIAsprint Frame Shield into the QIAsprint Prep Cover Frame before loading the QIAsprint Prep Cover. After the run, discard the QIAsprint Frame Shield.
- **Optional**: 14.3 M β-mercaptoethanol (β-ME, commercially available solutions are usually 14.3 M), alternatively dithiothreitol (DTT, 2 M stock solution), for purifying RNA from cell lines rich in RNases or from tissue
- QIAsprint Connect consumables (see Table 1)

* Do not use denatured alcohol, which contains other substances such as methanol or methyl ethyl ketone.

Table 1. QIAsprint Connect consumables

Item	Cat. no.	Pieces required for 4 × 96 samples
QIAsprint Prep Cover (8)	582103	1
QIAsprint Prep Plate Insert (32)	582226	4
QIAsprint Elution Plate (10)	583204	1

For cells

- Vortexer
- S-Blocks (24) (cat. no. 19585) or similar 96-well block

For tissue samples

- TissueLyser III or a similar device
- Collection Microtubes rack (10 × 96) (cat. no. 19560) and Collection Microtube Caps (120 × 8) (cat. no. 19566) or similar format for sample disruption
- Stainless Steel Beads, 5 mm (cat. no. 69989)

Important Notes

Determining the correct amount of starting material — cells

The minimum amount of starting material is approximately 100 cells. The maximum amount depends on the RNA content of the cell type but should not exceed 5 × 10^6 cells.

RNA content can vary greatly between cell types. The following examples illustrate how to determine the maximum amount of starting material:

- COS cells have high RNA content (approximately 35 µg RNA per 1 × 10^6 cells).
- HeLa cells have average RNA content (approximately 15 µg RNA per 1 × 10^6 cells).
- NIH/3T3 cells have low RNA content (approximately 10 µg RNA per 1 × 10^6 cells).

In general, we recommend starting with no more than 2–3 × 10^6 cells. Depending on RNA yield and purity, it may be possible to increase the cell number in subsequent preparations.

Counting cells is the most accurate way to quantify the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is shown in Table 2.

Table 2. Growth area and number of HeLa cells in various culture vessels

Cell-culture vessel	Growth area (cm^2)*	Number of cells†
Multiple plates		
96 wells	0.32–0.6	$4–5 \times 10^4$
48 wells	1	1×10^5
24 wells	2	2.5×10^5
12 wells	4	5×10^5
6 wells	9.5	1×10^6
Dishes		
35 mm		1×10^6
60 mm		2.5×10^6
100 mm		7×10^6
145–150 mm		2×10^7

* Per well, if multiwell plates are used; values may vary slightly depending on the supplier.
† Cell numbers are given for HeLa cells (average length, approx. 15 µm), assuming confluent growth. Cell numbers vary among animal cell types, which typically range from 10 to 30 µm in length.

Determining the correct amount of starting material — tissue

The maximum amount of tissue that can be processed depends on the RNA content of the tissue. In general, a maximum of 50 mg of tissue can be processed using the QIAsprint Connect procedure. If no information about the nature of the starting material is available, we recommend starting with no more than 10 mg of tissue.

Weighing tissue is the most accurate way to quantify the amount of starting material. However, the following guideline may be used: a 3 mm cube (volume = 27 mm^3) of most animal tissues weighs approximately 25–35 mg.

Handling and storage of starting material

RNA is not protected after harvesting until the sample is treated with RNAprotect® Cell Reagent (cultured cells only) or RNAprotect Tissue Reagent (animal tissues only), flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile may occur. It is therefore important that samples are either processed as soon as they are harvested, immediately immersed in RNAprotect Cell Reagent or RNAprotect Tissue Reagent, or immediately frozen in liquid nitrogen and stored at –90°C to –65°C (animal tissues only). Animal cells can be pelleted and stored at –90°C to –65°C until required for RNA purification.

An alternative to RNAprotect Tissue Reagent is Allprotect® Tissue Reagent, which provides immediate stabilization of DNA, RNA, and protein in tissue samples at room temperature. Because tissues stabilized with RNAprotect Tissue Reagent or Allprotect Tissue Reagent are partially dehydrated, a lower amount of starting material should be used.

Procedures for harvesting and RNA protection should be carried out as quickly as possible. Frozen samples must not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RLT, samples can be stored at –90°C to –65°C for several months.

Disruption and homogenization of starting material

Efficient disruption and homogenization of the starting material are required for all RNA purification procedures. Disruption and homogenization are two distinct steps:

- **Disruption**

  Complete disruption of the plasma membranes of cells and organelles is essential to release all RNA contained in the sample. Different sample types require different methods to achieve complete disruption. Incomplete disruption significantly reduces RNA yields.

- **Homogenization**

  Homogenization is necessary to reduce the viscosity of lysates generated during disruption. This process shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. In the QIAsprint RNA Tissue/Cells procedure, up to 1×10^6 cells can typically be processed by vortexing. If more cells must be processed, additional homogenization is required (see Table 3 on the facing page). Incomplete homogenization results in inefficient binding of RNA to magnetic particles, significantly reducing RNA yields.

Some disruption methods simultaneously disrupt and homogenize the sample, whereas others require an additional homogenization step. Table 3 provides an overview of different disruption and homogenization methods. This information can be used as a guide to select the appropriate method for the starting material.

Table 3. Guide to disruption and homogenization methods for samples

Sample	Disruption method	Homogenization method	Comments
Animal cells	Addition of lysis buffer	TissueRuptor® II or QIAshredder homogenizer or syringe and needle or vortexing (≤3 × 10^6 cells)	If processing ≤1 × 10^6 cells, lysate can be homogenized by vortexing.
Animal tissues	TissueLyser III or TissueLyser LT	TissueLyser III or TissueLyser LT	The TissueLyser III and TissueLyser LT give results comparable to using a rotor–stator homogenizer.
	TissueRuptor II	TissueRuptor II	Simultaneously disrupts and homogenizes
	Mortar and pestle	QIAshredder homogenizer or syringe and needle	The TissueRuptor II usually gives higher yields than mortar and pestle.

Disruption and Homogenization Using the TissueLyser III or TissueLyser LT

In bead milling, cells and tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur through the shearing and crushing action of the beads as they collide with the cells. Disruption efficiency is influenced by the following factors:

- Size and composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed and configuration of the TissueLyser III or TissueLyser LT
- Disintegration time

Stainless-steel beads with a diameter of 3–7 mm are optimal for use with animal tissues. All other disruption parameters must be determined empirically for each application. For other bead mills, refer to the suppliers' guidelines for further details.

Preparation of buffers

Preparation of Buffer RPE

Add the required volume of ethanol (96–100%) to the Buffer RPE concentrate, as described on the bottle. Tick the checkbox on the label to indicate that ethanol has been added. Store reconstituted Buffer RPE at room temperature (15–25°C). Reconstituted Buffer RPE is stable for up to 1 year when stored at room temperature, but only until the kit expiration date.

Preparation of DNase I stock solution (QIAsprint DNA Removal Set)

Prepare the DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µL of RNase-free water. In some cases, the DNase I vial may appear empty because the lyophilized enzyme adheres to the septum. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe, and mix gently by inverting the vial. Do not vortex.

Insoluble material may remain after dissolving DNase I; this does not affect DNase I performance. Owing to the production process, insoluble material may be present in the lyophilized enzyme. However, rigorous quality control tests are performed to ensure consistent DNase I activity from lot to lot.

Note: Do not vortex reconstituted DNase I, as it is sensitive to physical denaturation. Mix only by gently inverting the vial.

For long-term storage, remove the DNase I stock solution from the vial, divide it into single-use aliquots, and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 4 weeks. Do not refreeze aliquots after thawing.

Preparation of Buffer RDW (QIAsprint DNA Removal Set)

Add the required volume of isopropanol (96–100%) to Buffer RDW concentrate, as described on the bottle. Tick the checkbox on the label to indicate that isopropanol has been added. Store reconstituted Buffer RDW at room temperature (15–25°C). Reconstituted Buffer RDW is stable for up to 1 year when stored at room temperature, but only until the kit expiration date.

Note: Always mix reconstituted Buffer RDW by shaking before starting the purification procedure.

Preparing samples

The purification procedure is optimized for the extraction of total RNA including miRNA from up to 5 × 10^6 cells and 50 mg fresh/frozen tissue or 25 mg stabilized tissue.

Elution volumes and eluate handling

In the final step of the purification procedure, the RNA is eluted in RNase-free water. The protocol is optimized for a target eluate volume of 50–100 µL and requires the addition of 1 µL elution buffer per 1 µL magnetic particles to counteract the dead volume* of the magnetic particles. The default setting for the elution input volume is 100 µL, which results in an elution volume of approximately 80 µL. If the target elution volume is below 50 µL, reduction of the bead volume and adjustment of the input amount of starting material may be necessary.

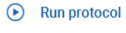
Storing RNA

If RNA is used directly in downstream applications, it can be kept on ice. For longer storage, RNA should be stored at –20°C or –70°C.

*Dead volume is the amount of liquid that cannot be recovered due to the inherent features of the magnetic particles.

Working with the QIAsprint Connect

Procedure on the QIAsprint Connect

1. Turn on the QIAsprint Connect instrument and log in.
2. Navigate to the Protocols screen with the tab in the upper part of the home screen.
3. Locate the desired protocol for the application, and press and 

.

   Optional: Enter run information in the Run details screen. This information will be displayed in the run report.
4. Close the hood and press **Confirm** to get to the loading screen.
5. With the plate hotels located on the lab bench, insert the plates according to the screen instructions into the hotels.
   - The QIAsprint Prep Cover must be located in the metal prep cover frame.

     Note: If desired, use the QIAsprint Frame Shield to minimize adhesions of beads to the QIAsprint Prep Cover Frame. For this, place QIAsprint Frame Shield into the QIAsprint Prep Cover Frame before loading the QIAsprint Prep Cover. After the run, discard the QIAsprint Frame Shield.
   - All QIAsprint Prep Plates need to sit firmly in their metal frames.
   - The QR codes need to face the user.
6. Open the hood and place the hotels on their platforms, taking care not to mix up the left and right hotels.
7. **Optional**: If you have heating or cooling steps during your protocol run and the thermal adapter is not already located on the heater–cooler, place the thermal adapter in one of the slots of the thermal adapter platform with the QR code facing the user. The instrument will install it automatically during the run.

8. Press **Start run** to start the run.
9. After the end of the run, remove the plate hotels from the instrument. Store the elution plate safely or use it directly in your downstream applications.

Protocol: Purification of RNA from Cells and Tissue Samples Including DNase Digestion

Important points before starting

- If using the QIAsprint RNA Tissue/Cells PrepSet, the QIAsprint DNA Removal Set, and QIAsprint Essential Kit B for the first time, read "Important Notes".
- After receiving the QIAsprint RNA Tissue/Cells PrepSet, the QIAsprint DNA Removal Set, and QIAsprint Essential Kit B, check the kits and components for damage. If any kit components are damaged, contact QIAGEN Technical Support or your local distributor. In the case of liquid spillage, refer to the Safety Information. Do not use damaged PrepSet and essential kit components because their use may lead to poor performance.

Things to do before starting

- Buffers RLT contains guanidine salt and is therefore not compatible with disinfecting reagents containing bleach.
- If necessary, redissolve any precipitate in Buffer RLT by warming.
- Equilibrate buffers to room temperature.
- All steps should be performed at room temperature. Work quickly.
- Add ethanol (96–100%) to Buffer RPE concentrate before use (see the bottle label for volume).
- If purifying RNA from cell lines rich in RNases or from tissue, we recommend adding either β-mercaptoethanol (β-ME) or 2 M dithiothreitol (DTT) to Buffer RLT before use (10 µL β-ME or 20 µL DTT per 1 mL Buffer RLT). Buffer RLT containing DTT or β-ME can be stored at room temperature for up to 1 month.

- In the following procedure, text marked with ● denotes RNA purification from cells and text marked with ▲ denotes RNA purification from tissue samples.
- Add the indicated volume of isopropanol to Buffer RDW provided with the QIAsprint DNA Removal Set (384) (see the bottle label for the required volume).
- Prepare the DNase I stock enzyme by adding 550 µL RNase-free water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme into appropriate portions and store at –30°C to –15°C for long-term storage. Avoid freezing and thawing more than three times. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.

Procedure for RNA purification from cells or tissue

1. ● Harvest up to 5 × 10^6 cells as a cell pellet or, for cells grown in a monolayer, aspirate and discard the cell culture medium from the cell culture vessel (up to 10 cm in diameter). Add 300 µL Buffer RLT to the pellet or directly to the cell culture vessel and homogenize. Transfer the lysate to a prep plate (lysate plate).

   ▲ Add 300 µL Buffer RLT to up to 50 mg frozen tissue or 25 mg stabilized tissue, then disrupt and homogenize. Transfer the lysate to a prep plate (lysate plate).

   Proceed with step 2.

   Note: See Table 3 for disruption and homogenization methods.

2. Prepare a master mix (x + 10% samples) by mixing 75 µL RNase-free water and 25 µL Proteinase K per sample. Mix thoroughly. Add 100 µL of the Proteinase K mix to each sample, mix thoroughly, and incubate for 10 min at room temperature. In the meantime, prepare the worktable as described in the next section.

Plates preparation

3. Prepare a master mix (x + 10% volume) by mixing 400 µL isopropanol and 20 µL MagG Bead Suspension per sample. Mix thoroughly. Add 420 µL of this mix to the sample.

   Note: Ensure sufficient resuspension of the MagG Bead Suspension before distribution by thorough vortexing.

4. Prepare the plate as described in the table below:

Plate	Content	Volume (µL)
Lysate Plate	Lysate supernatant MagG Bead Suspension Isopropanol 100%	400 20 400
Wash Plate 1	Buffer RPE	800
Wash Plate 2	Buffer RDW DNase	140 10
Wash Plate 3	Buffer RPE	500
Elution Plate	RNase-free water	100*

* Elution volume is set to 100 µL to receive approximately 80 µL. Volume can be adjusted. Recommended range is 50–200 µL.

Note: Residual adhesive can lead to instrument crash. If you cover plates with adhesive foil after plate preparation, ensure that no adhesive remains on the plate surface after removal of the foil.

5. Proceed with the procedure on the QIAsprint Connect (page 24) and the respective purification protocol.

Protocol: Purification of RNA from Cells and Tissue Samples

Important points before starting

- If using the QIAsprint RNA Tissue/Cells PrepSet and QIAsprint Essential Kit B for the first time, read "Important Notes".
- After receiving the QIAsprint RNA Tissue/Cells PrepSet, the QIAsprint DNA Removal Set, and QIAsprint Essential Kit B, check the kit and components for damage. If any kit components are damaged, contact QIAGEN Technical Support or your local distributor. In the case of liquid spillage, refer to the Safety Information. Do not use damaged PrepSet and essential kit components because their use may lead to poor performance.

Things to do before starting

- Buffers RLT contains guanidine salt and is therefore not compatible with disinfecting reagents containing bleach.
- If necessary, redissolve any precipitate in Buffer RLT by warming.
- Equilibrate buffers to room temperature.
- All steps should be performed at room temperature. Work quickly.
- Add ethanol (96–100%) to Buffer RPE concentrate before use (see the bottle label for volume).
- If purifying RNA from cell lines rich in RNases or from tissue, we recommend adding either β-ME or 2 M DTT to Buffer RLT before use (10 µL β-ME or 20 µL DTT per 1 mL Buffer RLT). Buffer RLT containing DTT or β-ME can be stored at room temperature for up to 1 month.

- In the following procedure, text marked with ● denotes RNA purification from cells and text marked with ▲ denotes RNA purification from tissue samples.

Procedure for RNA purification from cells or tissue

1. ● Harvest up to 5 × 10^6 cells as a cell pellet or, for cells grown in a monolayer, aspirate and discard the cell culture medium from the cell culture vessel (up to 10 cm in diameter). Add 300 µL Buffer RLT to the pellet or directly to the cell culture vessel and homogenize. Transfer the lysate to a prep plate (lysate plate).

   ▲ Add 300 µL Buffer RLT to up to 50 mg frozen tissue or 25 mg stabilized tissue, then disrupt and homogenize. Transfer the lysate to a prep plate (lysate plate).

   Proceed with step 2.

   Note: See Table 3 for disruption and homogenization methods.

2. Prepare a master mix (x + 10% samples) by mixing 75 µL RNase-free water and 25 µL Proteinase K per sample. Mix thoroughly. Add 100 µL of the Proteinase K mix to each sample, mix thoroughly, and incubate for 10 min at room temperature. In the meantime, prepare the worktable as described in the next section.

Plates preparation

3. Prepare a master mix (x + 10% volume) by mixing 400 µL isopropanol and 20 µL MagG Bead Suspension per sample. Mix thoroughly. Add 420 µL of this mix to the sample.

   Note: Ensure sufficient resuspension of the MagG Bead Suspension before distribution by thorough vortexing.

4. Prepare the plate as described in the table below:

Plate	Content	Volume (µL)
Lysate Plate	Lysate supernatant MagG Bead Suspension Isopropanol 100%	400 20 400
Wash Plate 1	Buffer RPE	800
Wash Plate 2	Buffer RPE	500
Elution Plate	RNase-free water	100*

* Elution volume is set to 100 µL to receive approximately 80 µL. Volume can be adjusted. Recommended range is 50–200 µL.

Elution volume is set to 100 µL to receive approximately 80 µL. Volume can be adjusted. Recommended range is 50–200 µL.

Note: Residual adhesive can lead to instrument crash. If you cover plates with adhesive foil after plate preparation, ensure that no adhesive remains on the plate surface after removal of the foil.

5. Proceed with the procedure on the QIAsprint Connect (page 24) and the respective purification protocol.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
General handling	
Error message in instrument	Refer to the *QIAsprint Connect User Manual* (**www.qiagen.com/HB-3708**).
Reagents loaded onto the worktable in wrong order	Ensure that all plate inserts holding lysate and wash buffers are loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.
RNA degraded	
Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in RNAprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –90°C to –65°C. Perform the procedure quickly, especially the first few steps. See "Handling and storage of starting material" (page 19).
RNase contamination	Although all buffers in the QIAsprint RNA Tissue/Cells PrepSet have been tested and are guaranteed to be RNase free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or during subsequent handling.
DNA contamination in downstream experiments	
Cell number too high	For some cell types, the efficiency of DNA removal may be reduced when processing very high cell numbers (containing more than 20 µg gDNA). If the eluted RNA contains substantial DNA contamination, try processing a smaller number of cells or perform a second DNase digestion of the eluted RNA followed by RNA cleanup.
Tissue has high DNA content	For certain tissues with extremely high DNA content (e.g., thymus), DNA may not be completely removed. Try using smaller samples (containing less than 20 µg gDNA) or perform a second DNase I digestion of the eluted RNA followed by RNA cleanup.

	Comments and suggestions
Beads in eluate	
Too much input material used	Adhere to the instructions regarding sample material, and carry out initial tests with lower amounts. Too much material may increase viscosity of the sample that can impact bead handling throughout the protocol.
Too much input material used	When using high amounts of sample material, more vigorous lysis such as the listed mechanical lysis options may be required to help with samples disruption and homogenization.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support** or contact one of the QIAGEN Technical Service Departments or local distributors (visit **support.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
QIAsprint RNA Tissue/Cells PrepSet (384)	For 384 RNA preparations from tissue or cell samples: Buffer RLT, Proteinase K, and RNase-free Water	586549
QIAsprint Essential Kit B (384)	For 384 preps for purification of nucleic acids: MagG Bead Suspension, Buffer RPE, and RNase-free Water	586009
QIAsprint DNA Removal Set (384)	DNase, RNase-free, 8 vials, 30 mL Buffer RDW, and 10 mL RNase-free water	586409
QIAsprint Prep Cover (8)	Set of 8 prep covers for use with QIAsprint Connect	582103
QIAsprint Prep Plate Insert (32)	Set of 32 prep plate inserts for use with QIAsprint Connect	582226
QIAsprint Elution Plate (10)	Set of 10 elution plates for use with QIAsprint Connect	583204
Related products		
QIAsprint Connect	Benchtop instrument for automated isolation of nucleic acids; includes 1 year warranty on parts and labor	9001234
QIAsprint Frame Shield (100)	Set of 100 frame shields for use with QIAsprint Connect	580010
QIAsprint Prep Cover Frame (2)	Set of 2 prep cover frames for use with QIAsprint Connect	582111
QIAsprint Prep Plate Frame (2)	Set of 2 prep plate frames for use with QIAsprint Connect	582211
Collection Microtubes (racked, 10 × 96)	Nonsterile polypropylene tubes (1.2 mL), 960 in racks of 96 for mechanical disruption and homogenization of the plant material	19560
Collection Microtube Caps (120 × 8)	Nonsterile polypropylene caps for collection microtubes (1.2 mL, 960 in strips of 8 for sealing the collection microtubes to prevent leakage	19566
TissueLyser III	Bead mill, 100–120/220–240 V, 50/60 Hz; requires TissueLyser Adapters (available separately)	9003240
TissueLyser LT	Compact bead mill, 100–240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately)	85600
TissueLyser Adapter Set 2 × 24	Two sets of adapter plates and two racks for use with 2 × 24 samples in 2 mL microcentrifuge tubes on the TissueLyser III	69982

Product	Contents	Cat. no.
TissueLyser Adapter Set 2 × 96	Two sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser III	69984
Plate Adapter Set	Two sets of adapter plates and two racks for use with two 96-well plates on the TissueLyser III	11990
2 mL Tube Holder Set	For sample homogenization of up to 2 × 48 samples in 2 mL bead tubes on the TissueLyser III	11993
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Buffer RLT	220 mL RNeasy Lysis Buffer	79216
RNAprotect Cell Reagent (250 mL)	250 mL RNAprotect Cell Reagent	76526
RNAprotect Tissue Reagent (250 mL)	250 mL RNAprotect Tissue Reagent for stabilization of RNA	76106
Allprotect Tissue Reagent (100 mL)	100 mL Allprotect Tissue Reagent for stabilization of DNA, RNA, and protein in tissue samples	76405

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Document Revision History

Date	Description
03/2026	Initial release

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