

QIASymphony SP Protocol Sheet

Purification of RNA from Tissues Using RNA_CT_400_V7, RNA_CT_800_V7, RNA_FT_400_V8, and miRNA_CT_400_V8

General information

These protocols are for purification of total RNA (>200 nt) or total RNA including miRNA (and other small RNAs) from animal and human tissues using the QIASymphony® SP and the QIASymphony RNA Kit.

Depending on the sample type, we recommend using either the standard or large-volume protocol. Select "RNA_CT_400_V7" (standard protocol) if processing up to 20 mg easy-to-lyse tissue or up to 10 mg thymus or spleen; select "RNA_CT_800_V7" (large-volume protocol) if processing up to 50 mg easy-to-lyse tissue; select "RNA_FT_400_V8" (fibrous tissue protocol) if processing up to 20 mg fibrous tissue; select "miRNA_CT_400_V8" to obtain total RNA including miRNA from up to 20 mg of tissue (up to 10 mg thymus or spleen).

Note: The RNA_FT_400_V8 and miRNA_CT_400_V8 protocols should be used with QIASymphony RNA Kits that have lot numbers higher than "145017065". When using kits with lot number "145017065" and lower, an inventory scan must be performed before first use with a new, unopened kit and the 200 µl elution volume cannot be used. If you have any questions, please contact QIAGEN Technical Services (see the back cover of our handbooks or visit www.qiagen.com).

Note: It is the user's responsibility to validate performance using this combination for any procedures used in their laboratory.

February 2013



Sample & Assay Technologies

Standard protocol

Kit	QIASymphony RNA Kit (cat. no. 931636)
Sample material	Up to 20 mg easy-to-lyse tissue or up to 10 mg thymus or spleen
Protocol name	RNA_CT_400_V7
Default Assay Control Set	ACS_RNA_CT_400_V7
Editable	Elution volume: 50 μ l, 100 μ l, 200 μ l
Required software version	Version 4.0 or higher

Large-volume protocol

Kit	QIASymphony RNA Kit (cat. no. 931636)
Sample material	Up to 50 mg easy-to-lyse tissue or up to 30 mg thymus or spleen
Protocol name	RNA_CT_800_V7
Default Assay Control Set	ACS_RNA_CT_800_V7
Editable	Elution volume: 100 μ l, 200 μ l
Required software version	Version 4.0 or higher

Fibrous tissue protocol

Kit	QIASymphony RNA Kit (cat. no. 931636)
Sample material	Up to 20 mg fibrous tissue
Protocol name	RNA_FT_400_V8
Default Assay Control Set	ACS_RNA_FT_400_V8
Editable	Elution volume: 50 μ l, 100 μ l, 200 μ l
Required software version	Version 4.0 or higher

miRNA protocol

Kit	QIASymphony RNA Kit (cat. no. 931636)
Sample material	Up to 20 mg easy-to-lyse or fibrous tissue or up to 10 mg thymus or spleen
Protocol name	miRNA_CT_400_V8
Default Assay Control Set	ACS_miRNA_CT_400_V8
Editable	Elution volume: 50 μ l, 100 μ l, 200 μ l
Required software version	Version 4.0 or higher

“Sample” drawer

Sample type	Animal and human tissue
Sample amount	Homogenate prepared from up to 50 mg of tissue, depending on protocol (see protocol tables above)
Lysate volume	400 μ l (standard, miRNA, and fibrous tissue protocols) 800 μ l (large-volume protocol)
Primary sample tubes	n/a
Secondary sample tubes	We recommend using 2 ml tubes (e.g., Sarstedt® cat. no. 72.693 or 72.608) or S-Blocks (cat. no. 19585). For a full list of compatible vessels, see www.qiagen.com/QIASymphony/Resources
Inserts	For more information, see the “Resources” tab at www.qiagen.com/QIASymphonyRNAKit

n/a = not applicable.

“Reagents and Consumables” drawer

Position A1 and/or A2	Reagent cartridge
Position B1	n/a
Tip rack holder 1–17	Disposable filter-tips, 200 μ l or 1500 μ l
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers
Tip racks slots 5 and 12	Accessory troughs for ethanol

n/a = not applicable.

“Waste” drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Empty liquid waste bottle

“Eluate” drawer

Elution rack (we recommend using slot 1, cooling position)	For more information, see the “Resources” tab at www.qiagen.com/QIAsymphonyRNAKit
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Required plasticware

	RNA_CT_400_V7		RNA_CT_800_V7		RNA_FT_400_V8		miRNA_CT_400_V8	
	24	96	24	48	24	96	24	72
Reagent cartridges	1	1	1	1	1	1	1	1 [§]
Sample prep cartridges*	21	84	21	42	21	84	21	63
8-Rod Covers[†]	3	12	3	6	3	12	3	9
Disposable filter-tips, 1500 µl[‡]	81	324	81	162	86	344	86	258
Disposable filter-tips, 200 µl[‡]	24	96	24	48	24	96	24	72
Ethanol (ml)	70	2 x 140	140	2 x 140	70	2 x 140	140	2 x 140

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 filter-tips/tip rack; the inventory scan requires additional tips (two 200 µl and seven 1500 µl tips).

§ 96 samples per reagent cartridge; 72 samples in one run.

Note: Numbers of filter-tips given may differ from the numbers displayed in the touchscreen depending on settings. We recommend loading the maximum possible number of tips.

Elution volume

The elution volume is selected in the touchscreen. Depending on the sample type and RNA content, the final eluate volume may vary by up to 15 µl less than the selected volume. Elution in smaller volumes increases the final RNA concentration, but reduces the yield and increases variability of the eluate volume. We recommend using the smallest elution volume only when the intended downstream application requires a higher RNA concentration.

Preparation of sample material

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.

Important points before starting

- For best results, stabilize animal tissues immediately in *RNAlater*[®] RNA Stabilization Reagent, or *Allprotect*[®] Tissue Reagent. Tissues can be stored in the reagent at 37°C for up to 1 day, at 15–25°C for up to 7 days, or at 2–8°C for up to 4 weeks (*RNAlater*) or 6 months (*Allprotect*). See the *RNAlater Handbook* or *Allprotect Tissue Reagent Handbook* for more information about the reagents and about stabilizing RNA in tissues.
- Buffer RLT Plus and buffers in the reagent cartridge contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 of the *QIASymphony RNA Handbook* for safety information. Take appropriate safety measures and wear gloves when handling Buffer RLT Plus and reagent cartridges.
- QIAGEN Proteinase K (cat. no. 19131) is required for the RNA_FT_400_V8 and miRNA_CT_400_V8 protocols, but is not supplied with the QIASymphony RNA Kit. It should be ordered separately.

Things to do before starting

- Buffer RLT Plus may form a precipitate upon storage. If necessary, redissolve by warming (37°C) and then place at room temperature (15–25°C).
- β -mercaptoethanol (β -ME) or dithiothreitol (DTT) must be added to Buffer RLT Plus before use to ensure RNA integrity.
If using β -ME, add 10 μ l β -ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. β -ME is stable in Buffer RLT Plus at room temperature for 1 month.
Alternatively, add 20 μ l of 2 M DTT per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- For isolation of total RNA including miRNA using the miRNA_CT_400_V8 protocol or the “RNA_FT_400_V8” protocol, transfer 2 ml proteinase K solution to each of the tubes in positions 3 and 4 of the enzyme rack on the reagent cartridge (see Table 5 on page 23 of the *QIASymphony RNA Handbook*).
Note: Do not use <2 ml of proteinase K with a new cartridge.
Tubes in positions 3 and 4 can remain empty with the lids on when using protocols that do not require proteinase K.
- Transfer 1.4 ml of DNase solution to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. For more information about preparation of DNase I, see the *QIASymphony RNA Handbook*, page 25.

- For tissues with high DNA content, such as spleen and thymus, we recommend using 1 vial of DNase I to obtain 1.4 ml of DNase solution. An additional RNase-Free DNase Set (see ordering information, *QIASymphony RNA Handbook*, page 42) will be required for each reagent cartridge used in that way.

Tissues

Fresh, frozen, or RNAlater or Allprotect stabilized tissue can be used for RNA purification. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen and immediately transfer to -70°C (remove tissue from stabilization reagent before freezing). Tissue can be stored for several months at -70°C . To process, do not allow unstabilized tissue to thaw during weighing or handling before disruption in Buffer RLT Plus. Homogenized tissue lysates (in Buffer RLT Plus, step 3 of the procedure) can also be stored at -70°C for several months. To process frozen lysates, thaw samples at room temperature ($15\text{--}25^{\circ}\text{C}$) or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C , which can cause chemical degradation of the RNA. Continue with step 4 of the procedure.

Pretreatment protocol for tissues

- 1. Excise the tissue sample from the animal or remove it from storage. Remove RNAlater or Allprotect stabilized tissues from the reagent using forceps. Remove any excess reagent. Do not use more than 20 mg tissue with the standard protocol or 50 mg with the high-volume protocol. Proceed immediately with step 2.**

Weighing tissue is the most accurate way to determine the amount.

Note: For tissues of high cell density, such as thymus and spleen, do not use more than 10 mg for the standard protocol, or 30 mg for the high-volume protocol.

- 2. Follow either step 2a or 2b, depending on how the tissues were stabilized.**

- 2a. For RNAlater or Allprotect stabilized tissues:**

If the entire piece of stabilized tissue can be used for RNA purification, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 3.

If only a portion of the stabilized tissue is to be used, place the tissue on a clean surface for cutting and cut it. Determine the weight of the piece to be used and place it into a suitably sized vessel for homogenization. Proceed with step 3.

RNA in the RNAlater or Allprotect stabilized tissue is still protected while the tissue is processed at $18\text{--}25^{\circ}\text{C}$. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNAlater or Allprotect reagent for further storage. Previously stabilized tissues can be stored at -70°C without the reagent.

2b. For unstabilized fresh or frozen tissues:

If the entire piece of tissue can be used for RNA purification, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately with step 3.

If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 3.

RNA in tissues is not protected after harvesting until the sample is treated with *RNAlater* or Allprotect reagent, flash frozen, or disrupted and homogenized in protocol step 3. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

Note: The remaining fresh tissue can be placed into *RNAlater* or Allprotect reagent for stabilization (see the *RNAlater Handbook* or the *Allprotect Tissue Reagent Handbook*). However, previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

3. Disrupt tissue and homogenize sample in Buffer RLT Plus. Disruption and homogenization of animal tissue can be performed by 2 alternative methods (3a or 3b).

See “Disruption and homogenization of starting material” starting on page 20 of the *QIASymphony RNA Handbook* for a more detailed description of disruption and homogenization methods. After storage in *RNAlater* RNA Stabilization Reagent or Allprotect Tissue Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem.

Note: Incomplete homogenization will lead to significantly reduced yields. Homogenization with rotor–stator homogenizers or the TissueLyser II generally results in higher total RNA yields than with other homogenization methods.

3a. TissueLyser II homogenization:

Place the weighed (fresh, frozen, or *RNAlater*-stabilized) tissue in a 2 ml microcentrifuge tube (not supplied), add 400 μ l Buffer RLT Plus (800 μ l for large-volume protocol), and add one stainless steel bead (3–7 mm diameter). Homogenize for 2–5 min at 25 Hz using the TissueLyser II. Rotate the TissueLyser rack, and homogenize for another 2–5 min at 25 Hz. Continue the protocol with step 4.

Note: The instructions in step 3a are only guidelines. They may need to be changed depending on the sample being processed and on the bead mill being used.

3a. TissueRuptor® homogenization:

Place the weighed (fresh, frozen, or stabilized in *RNAlater*) tissue in a 2 ml microcentrifuge tube (not supplied). Add 400 μ l Buffer RLT Plus (800 μ l for large-volume protocol). Homogenize immediately using the TissueRuptor with a disposable probe until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 4.

Homogenization with the TissueRuptor simultaneously disrupts and homogenizes the sample.

- 4. Centrifuge the tissue lysate for 3 min at maximum speed in a microcentrifuge. Carefully transfer the supernatant to sample tubes or plates that are compatible with the sample carrier of the QIASymphony SP.**

For a full list of compatible labware, see www.qiagen.com/QIASymphony/Resources. We recommend using 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, in the *QIASymphony RNA Handbook*, page 41).

In some preparations, very small amounts of insoluble material will be present, making the pellet invisible.

Lysates can stay on the instrument for 4–5 h at room temperature (15–25°C) without risk of RNA degradation.

- 5. For fatty tissues (e.g., brain, breast) only: add 100 µl chloroform to 400 µl lysate, and vortex thoroughly. Centrifuge at 4°C for 3 min at full speed (≥14,000 x g) to separate phases. Carefully transfer the aqueous phase to sample tubes or plates that are compatible with the sample rack of the QIASymphony SP.**
- 6. Place the tubes containing the lysates into the appropriate sample carrier, and load them into the “Sample” drawer.**
- 7. Begin the purification process, as described in the “General Purification Protocol”. See the *QIASymphony RNA Handbook*, page 24.**

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