QIAsymphony SP Protocol Sheet

Purification of RNA from Cells Using RNA_CT_400_V7, RNA_CT_800_V7, 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 cultured cells using the QIAsymphony® SP and the QIAsymphony RNA Kit.

Depending on the sample amount, we recommend using either the standard or large-volume protocol. In this procedure, you have a choice of 3 protocols. Select "RNA_CT_400_V7" (standard protocol) if processing ≤3 x 10^6 cells. Select "RNA_CT_800_V7" (large-volume protocol) if processing ≤1 x 10^7 cells. Finally, select "miRNA_CT_400_V8" to obtain total RNA, including miRNA and other small RNAs.

Note: The miRNA_CT_400_V8 protocol 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.

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Standard protocol

Kit	QIAsymphony RNA Kit (cat. no. 931636)
Sample material	≤3 x 10^6 cells
Protocol name	RNA_CT_400_V7
Default Assay Control Set	ACS_RNA_CT_400_V7
Editable	Elution volume: 50 µl, 100 µl, 200 µl
Required software version	Version 4.0 or higher

Large-volume protocol

Kit	QIAsymphony RNA Kit (cat. no. 931636)
Sample material	≤1 x 10^7 cells
Protocol name	RNA_CT_800_V7
Default Assay Control Set	ACS_RNA_CT_800_V7
Editable	Elution volume: 100 µl, 200 µl
Required software version	Version 4.0 or higher

miRNA protocol

Kit	QIAsymphony RNA Kit (cat. no. 931636)
Sample material	≤3 x 10^6 cells
Protocol name	miRNA_CT_400_V8
Default Assay Control Set	ACS_miRNA_CT_400_V8
Editable	Elution volume: 50 µl, 100 µl, 200 µl
Required software version	Version 4.0 or higher

"Sample" drawer

Sample type	Cultured cells
Sample amount	Lysate prepared from ≤3 x 10^6 cells (standard and miRNA protocols) or ≤1 x 10^7 cells (large-volume protocol)
Lysate volume	400 µl (standard and miRNA protocols) 800 µl (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 µl or 1500 µl
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

Required plasticware

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

- Cell pellets can be stored at –70°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that the pellets can be dislodged by flicking in step 2 of the procedure. Homogenized cell lysates (in Buffer RLT Plus, step 3 of the procedure) can be stored at –70°C for several months. To process frozen lysates, place samples at room temperature (15–25°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. If any insoluble material is visible, centrifuge for 5 minutes at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4 of the procedure.
- 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 miRNA_CT_400_V8 protocol, 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).
- Optional: β-mercaptoethanol (β-ME) may be added to Buffer RLT Plus before use to ensure RNA integrity. We do not recommend using β-ME except when purifying RNA from cells rich in RNases.
  If using β-ME, add 10 µl 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 µl of 2 M dithiothreitol (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, 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.

Pretreatment protocol for cells

1. Harvest cells according to steps 1a (for cells grown in suspension) or 1b (for cells grown in a monolayer).

1a. Cells grown in suspension (do not use more than 3 x 10^6 cells for the standard protocol or 1 x 10^7 cells for the large-volume protocol):

Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and continue with step 2 of the procedure.

Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

1b. Cells grown in a monolayer (do not use more than 3 x 10^6 cells for the standard protocol or 1 x 10^7 cells for the large-volume protocol):

Cells grown in a monolayer in cell-culture vessels can either be lysed directly in the culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet before lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

To lyse cells directly in culture dish:

Determine the number of cells. Completely aspirate the cell-culture medium, and continue immediately with step 2 of the procedure.

Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

To trypsinize cells:

Determine the number of cells. Aspirate the medium, and wash cells with PBS. Aspirate the PBS and add 0.10–0.25% trypsin in PBS to trypsinize the cells. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and pellet by centrifugation at 300 x *g* for 5 min. Completely aspirate the supernatant, and continue with step 2 of the procedure.

Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

2. **Disrupt cells by addition of Buffer RLT Plus. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 400 µl Buffer RLT Plus (800 µl for large-volume protocol). Vortex or pipet to mix, and proceed to step 3.**

   Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields.

   For direct lysis of cells grown in a monolayer, add 400 µl Buffer RLT Plus (800 µl for large-volume protocol) to the cell-culture dish. Collect cell lysate with a rubber cell scraper. Pipet lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

3. **Homogenize the lysate according to steps 3a or 3b.**

   One of two methods may be used to homogenize the lysate. See "Disruption and homogenization of starting material", *QIAsymphony RNA Handbook*, page 20, for a more detailed description of homogenization methods.

   If ≤1 x 10^6 cells are processed, the cells can be homogenized by vortexing for 1 min.

   Note: Incomplete homogenization can affect binding of nucleic acids to the magnetic particles and lead to significantly reduced yields.

3a. **Transfer the lysate to a 2 ml microcentrifuge tube, and add one stainless steel bead (5 mm diameter). Homogenize the lysate for 1 min at 20 Hz using the TissueLyser II. Rotate the TissueLyser rack, and homogenize for another 1 min at 20 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 bead mill being used.

3b. **Homogenize the lysate for 30 s using the TissueRuptor® or other rotor–stator homogenizer. Continue the protocol with step 4.**

4. **Transfer the homogenized lysates 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 (cat. no. 19585).

   If necessary, centrifuge briefly to reduce foaming. Avoid transfer of foam.

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

5. **Place the tubes containing the homogenized lysates into the appropriate sample carrier, and load them into the "Sample" drawer.**

6. **Begin the purification process, as described in the "General Purification Protocol". See the *QIAsymphony RNA Handbook*, page 24.**

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