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QIAsymphony® SP Protocol Sheet

Purification of RNA from FFPE sections using RNA_FFPE_130_V7

This document is the RNA_FFPE_130_V7 *QIAsymphony SP Protocol Sheet*, R2, for the QIAsymphony RNA Kit.

General information

The QIAsymphony RNA Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

This protocol is for purification of total RNA from FFPE tissue sections using the QIAsymphony SP and the QIAsymphony RNA Kit. The protocol recovers total RNA, including small RNA fragments. However, the protocol is not fully optimized for the isolation of miRNA. Depending on the type of tissue, miRNA recoveries may be lower than expected.

For purification of total RNA from biopsy needle core punches, go to the "Resources" tab at **www.qiagen.com/RNeasyFFPEKit** and select the relevant protocol from the list.

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

Kit	QIAsymphony RNA Kit (cat. no. 931636)
Sample material	FFPE tissue samples 5–20 µm thick
Protocol name	RNA_FFPE_130_V7
Default Assay Control Set	ACS_RNA_FFPE_130_V7
Editable	Elution volume: 50 µl, 100 µl, 200 µl
Required software version	Version 4.0 or higher

"Sample" drawer

Sample type	FFPE microtome sections
Sample amount	Lysate prepared from 1–2 sections 5–20 µm thick
Lysate volume	130 µl
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

	24 samples	**96 samples**
Reagent cartridges	1	2[§]
Sample prep cartridges*	15	45
8-Rod Covers[†]	3	9
Disposable filter-tips, 1500 µl[‡]	92	276
Disposable filter-tips, 200 µl[‡]	24	96
Ethanol (ml)	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).
§ 72 samples per reagent cartridge.

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 where 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 material safety data sheets (MSDSs), available from the product supplier.

Important point before starting

- Deparaffinization Solution (cat. no. 19093), QIAGEN® Proteinase K (cat. no. 19131), Buffer PKD (cat. no. 1034963), and DNase Booster Buffer (cat. no. 1064143) are required for the RNA_FFPE_130_V7 protocol, but are not supplied with the QIAsymphony RNA Kit. They should be ordered separately.

Things to do before starting

- 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.
- In the RNA_FFPE_130_V7 protocol, proteinase K is added in the manual pretreatment of the samples. Therefore, tubes in positions 3 and 4 can remain empty with the lids on.
- Transfer 2 ml DNase Booster Buffer to the tube in position 5 of the enzyme rack on the reagent cartridge.
- Set a thermal mixer or heated orbital incubator to 56°C for use in step 6.

Pretreatment protocol for FFPE sections

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections 5–20 µm thick.
3. If the sample surface has been exposed to air, discard the first 2–3 sections.

4. Immediately place the sections in a 2 ml sample tube compatible with the sample carrier of the QIAsymphony SP (not supplied).
5. Add 160 µl Deparaffinization Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.

   Deparaffinization Solution is not supplied with the QIAsymphony RNA Kit and should be ordered separately (cat. no. 19093).
6. Incubate at 56°C for 3 min, then allow to cool at room temperature.

   If samples turn waxy or solid upon cooling, add additional Deparaffinization Solution and repeat the 56°C incubation.
7. Add 120 µl Buffer PKD, and mix by vortexing.

   Buffer PKD is not supplied with the QIAsymphony RNA Kit and should be ordered separately (cat. no. 1034963).
8. Centrifuge for 1 min at 11,000 x *g* (10,000 rpm).
9. Add 10 µl proteinase K to the lower, uncolored phase. Mix gently by pipetting up and down.
10. Incubate at 56°C for 15 min.

    Note: Some particulate non-dissolved matter may remain after this step. It is not required to completely digest all sample material in order to achieve maximum RNA yields.
11. Place the tubes containing the digested samples into the appropriate sample carrier, and load them into the "Sample" drawer.
12. Begin the purification process, as described in the "General Purification Protocol". See the *QIAsymphony RNA Handbook*, page 24.

Revision history

Document revision history	
R2 12/2017	Update for QIAsymphony Software version 5.0

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