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QIAsymphony® SP Protocol Sheet

Tissue_LC_200_V7_DSP and Tissue_HC_200_V7_DSP

This document is the Tissue_LC_200_V7_DSP and Tissue_HC_200_V7_DSP *QIAsymphony SP Protocol Sheet*, R3, for QIAsymphony DSP DNA Mini Kit, version 1.

General information

The QIAsymphony DSP DNA Kit is intended for in vitro diagnostic use.

These protocols are for purification of total DNA from tissues and formalin-fixed, paraffin-embedded (FFPE) tissues using the QIAsymphony SP and the QIAsymphony DSP DNA Mini Kit.

Depending on the sample type, we recommend using either the low content (LC) or high content (HC) protocol. Tissues will provide increased DNA yields when processed with the high content protocol, but the low content protocol, in combination with a small elution volume (50 µl), may be used if high DNA concentration is required. For FFPE tissue we recommend using the low content protocol.

Low content protocol

Kit	QIAsymphony DSP DNA Mini Kit (cat. no. 937236)
Sample material	FFPE tissue and tissue* Up to 4 FFPE tissue sections, each with a thickness of up to 10 µm, or 8 sections, with a thickness of up to 5 µm and a surface area of up to 250 mm^2, can be combined in one preparation.
Protocol name	Tissue_LC_200_V7_DSP
Default Assay Control Set	ACS_Tissue_LC_200_V7_DSP
Elution volume	50 µl, 100 µl, 200 µl, or 400 µl
Required software version	Version 4.0 or higher

* See high content protocol for information about tissue samples.

High content protocol

Kit	QIAsymphony DSP DNA Mini Kit (cat. no. 937236)
Sample material	Tissue If information about the expected yield is not available, we recommend starting with 25 mg sample material. Depending on the yield obtained, the sample size can be increased in subsequent preparations.
Protocol name	Tissue_HC_200_V7_DSP
Default Assay Control Set	ACS_Tissue_HC_200_V7_DSP
Elution volume	100 µl, 200 µl, or 400 µl
Required software version	Version 4.0 or higher

Materials required but not provided

For all sample types

- Buffer ATL, 4 x 50 ml (cat. no. 939016)
- To minimize RNA content: DNase-free RNase A (stock solution of 100 mg/ml)

For FFPE tissue (xylene-free deparaffinization)

- Deparaffinization Solution (cat. no. 939018)

For FFPE tissue (deparaffinization using xylene)

- Xylene (99–100%)
- Ethanol (96–100%)*

"Sample" drawer

Sample type	FFPE tissue and tissue
Sample input volume	220 µl (required per sample, per protocol)†
Processed sample volume	200 µl
Primary sample tubes	n/a
Secondary sample tubes	See **www.qiagen.com/goto/dsphandbooks** for more information.
Inserts	Depends on type of sample tube used; for more information, see **www.qiagen.com/goto/dsphandbooks**.

† For both high and low content protocols, the system will not recognize if the sample volume is less than 220 µl because sample transfer is performed without liquid level detection. Therefore, make sure that the sample input volume is 220 µl.
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

n/a = not applicable.

* Do not use denatured alcohol, which contains additional substances such as methanol or methylethylketone.

"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)	See **www.qiagen.com/goto/dsphandbooks** for more information.

Required plasticware

Plasticware	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	26	50	74	98
Disposable filter-tips, 1500 µl†‡	72	136	200	264
Sample prep cartridges§	21	42	63	84
8-Rod Covers¶	3	6	9	12

* Using less than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/filter-tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

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

Elution volume is selected in the touchscreen. Depending on the sample type and DNA content, the final eluate volume may vary by up to 15 µl less than the selected volume. Due to the fact that the eluate volume might vary, we recommend checking the actual eluate volume when using an automated Assay Set System which does not verify the eluate volume prior to transfer. Elution in lower volumes increases the final DNA concentration, but slightly reduces the yield. We recommend using an elution volume appropriate for the intended downstream application.

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 point before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. In order to minimize RNA content in the sample, add RNase A to the sample in the step indicated in the respective pretreatment protocol.

Things to do before starting

- Check Buffer ATL for white precipitate. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Set a ThermoMixer® or shaker–incubator to the temperature required for the respective pretreatment.*

Tissues

Fresh and frozen tissue can be used for DNA purification. DNA yield and quality will depend on the tissue type, source, and storage conditions. Fresh tissue can be cut into small pieces and stored at –20°C or –80°C before processing. In general, we recommend using the high content protocol, which will provide increased DNA yields. The low content protocol, in combination with the 50 µl elution volume, is only recommended if high DNA concentrations are needed for downstream analysis. If information about the expected yield is not available, we recommend starting with 25 mg sample material using the high content protocol and the 200 µl elution volume. Depending on the yield obtained, the sample size can be increased or the elution volume can be decreased in subsequent preparations. Be aware that overloading preparations in combination with small elution volumes may cause carryover of magnetic particles into the eluate and could compromise DNA purity and downstream analysis.

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Pretreatment protocol for tissue

1. Transfer the tissue sample to a 2 ml microcentrifuge tube (not supplied).
2. Add 220 µl Buffer ATL.
3. Add 20 µl proteinase K and mix by tapping the tube.

   Note: Use proteinase K from the enzyme rack of the QIAsymphony DSP DNA Mini Kit.
4. Place the tube in a ThermoMixer or shaker–incubator and incubate at 56°C with shaking at 900 rpm until the tissue is completely lysed.

   Note: Lysis time varies depending on the tissue type processed. For most tissues, lysis is completed within 3 hours. If lysis is incomplete after 3 hours, as indicated by the presence of insoluble material or highly viscous lysates, lysis time can be prolonged or insoluble material can be removed by centrifugation as described in step 6. Overnight lysis is possible and does not affect the preparation.
5. To minimize RNA content in the sample, add 4 µl RNase A (100 mg/ml) and incubate for 2 minutes at room temperature (15–25°C) before continuing with step 6.
6. Homogenize the sample by pipetting up and down several times.

   Note: If pieces of insoluble material are still present, centrifuge at 3000 x *g* for 1 minute.
7. Carefully transfer 220 µl of the supernatant to sample tubes that are compatible with the sample carrier of the QIAsymphony SP.

   For a full list of compatible sample tubes, see **www.qiagen.com/goto/dsphandbooks**. We recommend using 2 ml tubes (e.g., Sarstedt® cat. no. 72.693 or 72.608).

FFPE tissue

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation of nucleic acids. To limit the extent of DNA fragmentation, be sure to:

- Fix tissue samples in 4–10% formalin as quickly as possible after surgical removal
- Use a fixation time of 14–24 hours (longer fixation times lead to more severe DNA fragmentation, resulting in poor performance in downstream assays)
- Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit the proteinase K digestion)

Starting material for DNA purification should be freshly cut sections of FFPE tissue. Up to 4 sections, each with a thickness of up to 10 µm, or 8 sections with a thickness of up to 5 µm and a surface area of up to 250 mm^2, can be processed in one preparation. If information about the nature of your starting material is not available, we recommend starting with no more than 3 sections in a

single preparation. Depending on DNA yield and purity, it may be possible to use up to 8 sections in subsequent preparations.

Note: The FFPE tissue protocols are specially designed to only copurify low amounts of RNA. This will lead to a reduced photometric measurement value compared to values obtained with the manual QIAamp® DSP DNA FFPE Tissue kit.

Pretreatment protocol for FFPE tissue

Method 1: deparaffinization using Deparaffinization Solution

1. Using a scalpel, trim excess paraffin from the sample block.
2. Cut up to 4 sections 10 µm thick, or up to 8 sections 5 µm thick.

   Note: If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Immediately place the sections in a 2 ml Sarstedt tube (not supplied, cat. no. 72.693 or 72.608) that is compatible with the sample carrier of the QIAsymphony SP.
4. Add 200 µl Buffer ATL to the sections.
5. Add 20 µl proteinase K.

   Note: Use proteinase K from the enzyme rack of the QIAsymphony DSP DNA Mini Kit.
6. Add 160 µl or 320 µl Deparaffinization Solution (see table below) and mix by vortexing.

Thickness of sections	Number of sections	Volume of Deparaffinization solution
5 µm	1–4	160 µl
	5–8	320 µl
10 µm	1–2	160 µl
	3–4	320 µl

7. Place the tube in a ThermoMixer or shaker–incubator and incubate at 56°C for 1 hour with shaking at 1000 rpm until the tissue is completely lysed.

   Note: Lysis time varies depending on the tissue type processed. For most tissues, lysis is completed within 1 hour. If lysis is incomplete after 1 hour, as indicated by the presence of insoluble material, lysis time can be prolonged or insoluble material can be pelleted by centrifugation as described in step 10. Overnight lysis is possible and does not affect the preparation.

8. Incubate at 90°C for 1 hour.

   Note: The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block reaches 90°C.

9. To minimize RNA content in the sample, add 2 µl RNase A (100 mg/ml) to the lower phase and incubate for 2 minutes at room temperature before continuing with step 10. Allow the sample to cool to room temperature before adding RNase A.
10. Centrifuge at full speed for 1 minute at room temperature.
11. Carefully transfer tubes (containing both phases) to the sample carrier of the QIAsymphony SP.

Method 2: deparaffinization using xylene

1. Using a scalpel, trim excess paraffin from the sample block.
2. Cut up to 4 sections 10 µm thick, or up to 8 sections 5 µm thick.

   Note: If the sample surface has been exposed to air, discard the first 2–3 sections.

3. Immediately place the sections in a 1.5 or 2 ml microcentrifuge tube (not supplied) and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 seconds.
4. Centrifuge at full speed for 2 minutes at room temperature.
5. Remove the supernatant by pipetting. Do not remove any of the pellet.
6. Add 1 ml ethanol (96–100%) to the pellet and mix by vortexing.

   Note: The ethanol extracts residual xylene from the sample.

7. Centrifuge at full speed for 2 minutes at room temperature.
8. Remove the supernatant by pipetting. Do not remove any of the pellet.

   Note: Carefully remove any residual ethanol using a fine pipet tip.

9. Open the tube and incubate at room temperature (15–25°C) for 10 minutes or until all residual ethanol has evaporated.

   Note: Incubation may be performed at temperatures up to 37°C.

10. Resuspend the pellet in 220 µl Buffer ATL.
11. Add 20 µl proteinase K and mix by vortexing.

    Note: Use proteinase K from the enzyme rack of the QIAsymphony DSP DNA Mini Kit.

12. Incubate at 56°C for 1 hour (or until the sample has been completely lysed).

    Note: Lysis time varies depending on the tissue type processed. For most tissues, lysis is completed within 1 hour. If lysis is incomplete after 1 hour, as indicated by the presence of insoluble material, lysis time can be prolonged or insoluble material can be removed by centrifugation as described in step 16. Overnight lysis is possible and does not affect the preparation.

13. Incubate at 90°C for 1 hour.

    Note: The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block reaches 90°C.

14. Briefly centrifuge the sample to remove drops from the inside of the lid.

15. To minimize RNA content in the sample, add 2 µl RNase A (100 mg/ml) and incubate for 2 minutes at room temperature before continuing with step 16. Allow the sample to cool to room temperature before adding RNase A.

16. Carefully transfer 220 µl of the lysate to sample tubes that are compatible with the sample carrier of the QIAsymphony SP.

    Note: If lysates contain undigested material, centrifuge at full speed for 2 minutes at room temperature before transferring the supernatant to sample tubes. For a full list of compatible sample tubes, see **www.qiagen.com/goto/dsphandbooks**. We recommend use of 2 ml tubes (e.g., Sarstedt, cat. no. 72.693 or 72.608).

Revision history

Document revision history	
R3 12/2017	Update for QIAsymphony Software version 5.0

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN® kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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