August 2015

QIAsymphony® SP Protocol Sheet

Tissue_LC_200_V7_DSP and Tissue_HC_200_V7_DSP (user-validated for QIAsymphony DSP DNA Mini Kit)

This document is the *Tissue_LC_200_V7_DSP and Tissue_HC_200_V7_DSP (user-validated for QIAsymphony DSP DNA Mini Kit)* QIAsymphony SP Protocol Sheet, R1, for Kit Version 1.

General information

These protocols are for purification of total DNA from cultured cells and bacterial cultures 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. Cultured cells and bacterial cultures 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.

The QIAsymphony DSP DNA Mini Kit, in combination with the Tissue_LC_200_V7_DSP and Tissue_HC_200_V7_DSP (user-validated for QIAsymphony DSP DNA Mini Kit) protocols for purification of total DNA from cultured cells and bacterial cultures, is intended for molecular biology applications. The product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Low content protocol

Kit	QIAsymphony DSP DNA Mini Kit (cat. no. 937236)
Sample material	Cultured cells and bacterial cultures Recommended maximum sample sizes: For cell culture, 5 x 10^6 cells For bacteria, 1 x 10^9 cells
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

High content protocol

Kit	QIAsymphony DSP DNA Mini Kit (cat. no. 937236)
Sample material	Cultured cells and bacterial cultures Recommended maximum sample sizes: For cell culture, 1 x 10^7 cells For bacteria, 4 x 10^9 cells
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

Materials required but not provided

For all sample types

- To minimize RNA content: RNase A (stock solution of 100 mg/ml) (cat. no. 19101)

For Gram-negative bacteria

- Buffer ATL (cat. no. 19076)

For Gram-positive bacteria

- Buffer P1 (cat. no. 19051)
- Lysozyme (stock solution of 100 mg/ml)

For cultured cells

- Buffer P1 (cat. no. 19051)

"Sample" drawer

Sample type	Cultured cells and bacterial cultures
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.

"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

- If using Buffer ATL, check that it does not contain 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.*

Cultured cells

Both fresh and frozen cultured cells may be used. We recommend using the high content protocol for up to 1 x 10^7 cells. The low content protocol will result in lower DNA yields and is only recommended, in combination with a small elution volume (50 µl), if high DNA concentration is required. Frozen cell pellets should be resuspended in Buffer P1 as described in the pretreatment protocol.

Pretreatment protocol for cultured cells

1. Centrifuge a maximum 1 x 10^7 cells at 300 x *g* for 5 minutes at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the cell pellet.

   Note: The cell pellet can be stored at –20°C or –70°C for future use, or can be used immediately.
2. Resuspend the pellet in 220 µl Buffer P1 and transfer the sample to a 2 ml microcentrifuge tube (not supplied).
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 for 30 minutes to 2 hours.

   Note: Lysis time depends on the type of cells and cell number. If lysis is incomplete after 2 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.

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

6. 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 into sample tubes. 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).

Bacteria

Both fresh and frozen bacterial cultures may be used. We recommend using the high content protocol with up to 4 x 10^9 cells. The low content protocol will result in lower DNA yields and is only recommended, in combination with small elution volume (50 µl), if high DNA concentration is required. Bacterial growth is usually measured as optical density (OD) of the bacterial culture using a spectrophotometer. However, OD readings strongly depend on the type of spectrophotometer used and the bacterial species measured. We therefore recommend calibrating the spectrophotometer by correlating measured ODs to bacterial cell numbers. Frozen pellets should be resuspended in Buffer P1 (Gram-positive bacteria) or Buffer ATL (Gram-negative bacteria), as described in the pretreatment protocols.

Pretreatment protocol for Gram-negative bacteria

1. Harvest a maximum of 4 x 10^9 cells by centrifugation for 10 minutes at 5000 x *g* at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the bacterial pellet.

   Note: The cell pellet can be stored at –20°C or –70°C for future use, or can be used immediately.
2. Resuspend the bacterial pellet in 220 µl Buffer ATL and transfer the sample to a 2 ml microcentrifuge tube (not supplied).
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 for 30 minutes to 2 hours.

   Note: Lysis time depends on the type of cells and cell number. If lysis is incomplete after 2 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.
5. To minimize RNA content in the sample, add 4 µl RNase A (100 mg/ml) and incubate for 2 minutes at room temperature before continuing with step 6.

6. 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 into sample tubes. 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).

Pretreatment protocol for Gram-positive bacteria

1. Harvest a maximum of 4 x 10^9 cells by centrifugation for 10 minutes at 5000 x *g* at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the bacterial pellet.

   Note: The cell pellet can be stored at –20°C or –70°C for future use, or can be used immediately.
2. Resuspend the bacterial pellet in 200 µl Buffer P1 and transfer the sample to a 2 ml microcentrifuge tube (not supplied).
3. Add 20 µl lysozyme (100 mg/ml) and mix by tapping the tube.
4. Place the tube in a ThermoMixer or shaker–incubator and incubate at 37°C with shaking at 900 rpm for 30 minutes to 2 hours.

   Note: Lysis time depends on the type of cells and cell number.
5. 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.
6. Incubate at 56°C with shaking at 900 rpm for 30 minutes.
7. To minimize RNA content in the sample, add 4 µl RNase A (100 mg/ml) and incubate for 2 minutes at room temperature before continuing with step 8.
8. 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 into sample tubes. 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).

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.