October 2022

Supplementary Protocol for User Self-Validation

QIAsymphony® DSP DNA Mini Kit

Tissue_LC_200_V7_DSP and Tissue_HC_200_V7_DSP; Version 2

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 (cat. no. 937236).

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 this Supplementary Protocols for User Self-Validation is for purification of total DNA from cultured cells and bacterial cultures.

Note: It is the user's responsibility to validate performance using this combination for any procedures used in their laboratory according to local requirements, laws, and regulations.

Equipment and reagents to be supplied by the user

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.

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)

Warnings and precautions

Read all instructions carefully before using the kit.

Please be aware of following remaining risks:

When using secondary tubes, please ensure that the sample IDs are not mixed up during transfer of Sample ID from primary to secondary tube.

Sample IDs can also be entered manually (for details refer to the *QIAsymphony SP User Manual*). If wrong ID data are entered manually, wrong correlation between sample and result can occur.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view and print the SDS for each QIAGEN kit and kit component.

All chemicals and biological materials are potentially hazardous. Specimens and samples are potentially infectious and must be treated as biohazardous materials.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers in the reagent cartridge (RC) contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Emergency information

CHEMTREC
USA & Canada 1-800-424-9300
Outside USA & Canada +1 703-527-3887

Precautions

The following hazard and precautionary statements apply to components of QIAsymphony DSP DNA Kits.

QSB1

Contains: guanidine thiocyanate; isopropanol. Danger! May be harmful if swallowed or in contact with skin. May be harmful if swallowed and enters airways. Causes severe skin burns and eye damage. May cause drowsiness or dizziness. Flammable liquid and vapor. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor/ physician. Rinse mouth. Do NOT induce vomiting. Wash contaminated clothing before reuse. Store in a well-ventilated place. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

MBS

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Proteinase K

Contains: proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray. Wear protective gloves/ protective clothing/ eye protection/ face protection. Wear respiratory protection. IF exposed or concerned: Call a POISON CENTER or doctor/ physician. Remove person to fresh air and keep comfortable for breathing. Dispose of contents/ container to an approved waste disposal plant.

QSL1

Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. May cause an allergic skin reaction. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QSW1

Contains: ethanol; guanidine hydrochloride; lithium chloride. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Flammable liquid and vapor. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/ protective clothing/ eye protection/ face protection. Call a POISON CENTER or doctor/ physician if you feel unwell. Take off contaminated clothing and wash before reuse. Store in a well-ventilated place. Dispose of contents/ container to an approved waste disposal plant.

QSW2

Contains: ethanol. Danger! Causes serious eye irritation. Highly flammable liquid and vapor. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/ protective clothing/ eye protection/ face protection. Store in a well-ventilated place. Dispose of contents/ container to an approved waste disposal plant.

General information

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
Editable	Eluate volumes: 50, 100, 200, or 400 µL
Required software version	Version 4.0 or higher

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
Editable	Eluate volumes: 100, 200, or 400 µL
Required software version	Version 4.0 or higher

"Sample" drawer

Sample type	Cultured cells and bacterial cultures
Sample input volume	220 µL (required per sample, per protocol)*
Processed sample volume	220 µL
Primary sample tubes	n/a
Secondary sample tubes	See the labware list that can be found under the resource tab of the product page on **www.qiagen.com** for more information.
Inserts	Depends on type of sample tube used; see **www.qiagen.com** for more information.

* 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 (RC)
Position B1	n/a
Tip rack holder 1–17	Disposable filter-tips, 200 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	Liquid waste bottle

“Eluate” drawer

Elution rack (we recommend using slot 1, cooling position)	For more information, see the labware list that can be found under the resource tab of the product page on **www.qiagen.com**.

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/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 on the touchscreen depending on settings, for example, number of internal controls used per batch.

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.

Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, the quality of the starting material, and the protocol used for isolation of DNA. Elution in smaller volumes increases the final DNA concentration in the eluate but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application. QIAsymphony DSP DNA Kits copurify RNA and DNA if both are present in the sample. To minimize RNA content in the sample, add RNase A to the sample in the step indicated in the respective pretreatment protocol.

Storing DNA

Storage conditions and duration of the purified nucleic acid depends on the sample material used.

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been established for the QIAsymphony DSP DNA Kits in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

Reagent storage and handling

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

QIAsymphony DSP DNA Kits should be stored upright at room temperature (15–25°C). The magnetic particles in the reagent cartridges (RC) remain active when stored at this temperature. When stored properly, the kit is stable until the expiration date on the kit box.

QIAsymphony DSP DNA Kits contain ready-to-use proteinase K solution that can be stored at room temperature.

Note: The label on the QIAsymphony DSP DNA Kit box displays the expiration date of the kit. The result file documents the expiration dates for only the reagent cartridge (RC).

In-use stability

Partially used reagent cartridges (RC) can be stored for a maximum of 4 weeks, upright at room temperature (15–25°C), enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge (RC) is partially used, replace the cover of the trough containing the magnetic particles and seal the reagent cartridge (RC) with the provided Reuse Seal Strips immediately after the end of the protocol run to avoid evaporation.

To avoid reagent evaporation, the reagent cartridge (RC) should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 32°C.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge (RC) is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Avoid exposure of the reagent cartridges (RC) to UV light (e.g., used for decontamination) as exposure may cause accelerated aging of the reagent cartridges (RC) and buffers.

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.

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 min 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 min to 2 h.

   Note: Lysis time depends on the type of cells and cell number. If lysis is incomplete after 2 h, 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 min at room temperature (15–25°C) 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 min at room temperature before transferring the supernatant into sample tubes. For a full list of compatible sample tubes, see the labware list that can be found under the Resource tab of the product page on **www.qiagen.com** for more information. 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 min 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 min to 2 h.

   Note: Lysis time depends on the type of cells and cell number. If lysis is incomplete after 2 h, 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 min 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 min at room temperature before transferring the supernatant into sample tubes. For a full list of compatible sample tubes, see the labware list that can be found under the **Resource** tab of the product page on **www.qiagen.com** for more information. 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 min 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 min to 2 h.

   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 min.
7. To minimize RNA content in the sample, add 4 µL RNase A (100 mg/mL) and incubate for 2 min 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 min at room temperature before transferring the supernatant into sample tubes. For a full list of compatible sample tubes, the labware list that can be found under the **Resource** tab of the product page on **www.qiagen.com** for more information. We recommend using 2 mL tubes (e.g., Sarstedt cat. no. 72.693 or 72.608).

Protocol

Important point before starting

- Make sure that you are familiar with operating the QIAsymphony SP. Refer to the user manuals supplied with your instrument for operating instructions.
- Optional maintenance is not mandatory for instrument function, but is highly recommended to reduce risk of contamination.
- Make you are familiar with this Supplementary Protocol for User Self-Validation (available at **www.qiagen.com**).
- Before using a reagent cartridge for the first time, check that Buffers QSL1 and QSB1 do not contain any precipitates. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitates. Make sure to replace the troughs in the correct positions. If the reagent cartridge is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.
- Try to avoid vigorous shaking of the reagent cartridge (RC) otherwise foam may be generated, which can lead to liquid-level detection problems.

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.*
- Before starting the procedure, make sure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Make sure that the piercing lid is placed on the reagent cartridge and the lid of the magnetic-particle trough has been removed or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- Make sure to open the enzyme tubes.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.
- For information about sample tubes compatible with a certain protocol, see the corresponding labware list (available at **www.qiagen.com**).
- For information about minimum sample volumes for samples in primary and secondary tubes for a certain protocol, see the corresponding labware list (available at **www.qiagen.com**). This information also indicates which tubes can be used for different protocols.

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

Procedure

1. Close all drawers and the hood.
2. Power ON the QIAsymphony SP, and wait until the **Sample Preparation** screen appears and the initialization procedure has finished.
3. The power switch is located at the bottom, left corner of the QIAsymphony SP.
4. Log on to the instrument.
5. Make sure the "Waste" drawer is properly prepared and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.
6. Load the required elution rack into the "Eluate" drawer.

   Do not load a 96-well plate onto "Elution slot 4".

   "Elution slot 1", with the corresponding cooling adapter, must be used.

   When using a 96-well plate, make sure that the plate is in the correct orientation, as incorrect placement may cause sample mix-up in downstream analysis.

   When using the Elution Microtubes CL rack, remove the bottom by twisting the rack until the bottom comes off.
7. Load the required reagent cartridge(s) and consumables into the "Reagents and Consumables" drawer.
8. Perform an inventory scan of the "Reagents and Consumables" drawer.
9. Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.

   Note: To ensure correct liquid aspiration, push the tubes and inserts down to the bottom of the tube carrier
10. Using the touchscreen, enter the required information for each batch of samples to be processed.

    Enter the following information:

    10a. Sample information (depending on sample racks used)
    10b. Protocol to be run (Assay Control Set)
    10c. Elution volume and output position
    After information about the batch has been entered, the status changes from **LOADED** to **QUEUED**. As soon as one batch is queued the Run button appears.
11. Press the Run button to start the purification procedure.

    All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from **RUNNING** to **COMPLETED**.
12. Retrieve the elution rack containing the purified nucleic acids from the "Eluate" drawer.
13. The DNA is ready to use or can be stored. Details are given in the relevant protocol sheets available at **www.qiagen.com**

    We recommend removing the eluate plate from the "Eluate" drawer immediately after the run has finished. Depending on temperature and humidity, elution plates left in the QIAsymphony SP after the run is completed may experience condensation or evaporation.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications.

    If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnetic rack and the eluates transferred to a clean tube.

    Result files are generated for each elution plate.

14. If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing proteinase K with screw caps immediately after the end of the protocol run to avoid evaporation.

    Note: For more information about storage of partially used reagent cartridges (RC), see “Reagent storage and handling” page 6.

15. Discard used sample tubes and waste according to your local safety regulations.

    See page 2 for Safety information.

16. Clean the QIAsymphony SP.

    Follow the maintenance instructions in the user manuals supplied with your instrument. Make sure to clean the tip guards regularly to minimize the risk of cross-contamination.

17. Close the instrument drawers and power OFF the QIAsymphony SP.

Document Revision History

Date	Changes
10/2022	Version 2, Revision 1 • Creation of document

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.