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QIAsymphony® SP Protocol Sheet

DNA_Buffy_Coat_200_V7 DSP protocol

This document is the DNA_Buffy_Coat_200_V7 DSP *QIAsymphony SP Protocol Sheet*, R2, for QIAsymphony DSP DNA Mini Kit, version 1.

General information

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

This protocol is for purification of total genomic and mitochondrial DNA from fresh or frozen buffy coat using the QIAsymphony SP and the QIAsymphony DSP DNA Mini Kit.

Kit	QIAsymphony DSP DNA Mini Kit (cat. no. 937236)
Sample material	Buffy coat (EDTA, citrate, or heparin anti-coagulated)
Protocol name	DNA_BC_200_V7_DSP
Default Assay Control Set	ACS_BC_200_V7_DSP
Editable	Elution volume: 200 µl, 300 µl, 400 µl
Required software version	Version 4.0 or higher

"Sample" drawer

Sample type	Buffy coat (EDTA, citrate, or heparin anti-coagulated)
Sample volume	Depends on type of sample tube used; for more information see **www.qiagen.com/goto/dsphandbooks**.
Primary sample tubes	n/a
Secondary sample tubes	For more information see **www.qiagen.com/goto/dsphandbooks**.
Inserts	Depends on type of sample tube used; for more information see **www.qiagen.com/goto/dsphandbooks**.

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)	For more information see www.qiagen.com/goto/dsphandbooks.

Required plasticware

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	2	2	2	2
Disposable filter-tips, 1500 µl†‡	110	212	314	416
Sample prep cartridges§	18	36	54	72
8-Rod Covers¶	3	6	9	12

* Use of 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 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 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 may vary, we recommend checking the actual eluate volume when using an automated assay setup system that 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 may copurify RNA if it is present in the sample. In order to minimize RNA content in the sample, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml.

Buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. The efficiency of leukocyte enrichment depends on the procedure used to prepare buffy coat and on the accuracy with which the buffy coat layer is extracted. Prepare buffy coat by centrifuging whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) at 900–1100 x *g* for 10 minutes at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Approximately 1 ml leukocyte-containing fraction should be harvested from 10 ml centrifuged whole blood, which, on average, gives a 5–6x enrichment. For example, 10 ml whole blood with a white blood cell count of 6 x 10^6 cells/ml results in 1 ml buffy coat. Assuming a 5x enrichment of white blood cells, this results in 3 x 10^7 cells/ml. Therefore, in a protocol that uses 200 µl buffy coat, 6 x 10^6 cells will be used.

To avoid overloading the DNA purification procedure, do not prepare buffy coat samples of >10x enrichment. If buffy coat samples are of >10x enrichment, dilute the samples to 10x enrichment or less with PBS or use less starting material in the DNA purification procedure.

Buffy coat samples may be used immediately or stored at –20°C or –70°C for purification of DNA at a later date. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. To ensure reliable sample transfer, avoid generating foam in sample tubes. Try to avoid blood clots in the samples and, if necessary, transfer the sample without clots to a fresh tube.

Revision history

Document Revision History	
R2 12/2017	Update for QIAsymphony Software version 5.0

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