QIAsymphony® SP Protocol Sheet

Cellfree200_V7_DSP protocol

Version management

This document is the QIAsymphony Cellfree200_V7_DSP Protocol Sheet, Version 1, R1.

General information

For in vitro diagnostic use.

Kit	QIAsymphony DSP Virus/Pathogen Mini Kit
Sample material*	Plasma, serum, and CSF
Protocol name	Cellfree200_V7_DSP
Default Assay Control Set	ACS_Cellfree200_V7_DSP_default_IC
Editable	Eluate volume: 60 µl, 85 µl, 110 µl
Required software version	Version 4.0

* For additional information see “Preparation of sample material” and “Limitations”, page 6.

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Sample & Assay Technologies

“Sample” drawer

Sample type	Plasma, serum, and CSF
Sample volume	Depends on type of sample tube used; for more information see www.qiagen.com/goto/dsphandbooks
Primary sample tubes	See www.qiagen.com/goto/dsphandbooks for more information
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
Other	Carrier RNA–Buffer AVE mix required; use of internal control is optional

“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 µl
Tip rack holder 1–17	Disposable filter-tips, 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges
Unit box holder 1–4	Unit boxes containing 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)	See www.qiagen.com/goto/dsphandbooks for more information

Required plasticware

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 μl[†‡]	30	54	78	102
Disposable filter-tips, 1500 μl[†‡]	101	182	271	354
Sample prep cartridges[§]	21	42	63	84
8-Rod Covers[¶]	3	6	9	12

* Use of more than one internal control per batch and performing more than one inventory scan requires additional disposable filter-tips. 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, for example, number of internal controls used per batch.

Selected elution volume

Selected elution volume (μl)*	Initial elution volume (μl)†
60	90
85	115
110	140

* The elution volume selected in the touchscreen. This is the minimum accessible volume of eluate in the final elution tube.

† The initial volume of elution solution required to ensure that the actual volume of eluate is the same as the selected volume.

Preparation of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture

Selected elution volume (μl)	Volume stock carrier RNA (CARRIER) (μl)	Volume internal control (μl)*	Volume Buffer AVE (AVE) (μl)	Final volume per sample (μl)
60	2.5	9	108.5	120
85	2.5	11.5	106	120
110	2.5	14	103.5	120

* The calculation of the amount of internal control is based on the initial elution volumes. Additional void volume depends on the type of sample tube used; see www.qiagen.com/goto/dsphandbooks for more information.

Note: The values displayed in the table are for preparation of internal control–carrier RNA (CARRIER) mixture for a downstream assay that requires 0.1 μl internal control/μl eluate.

Tubes containing internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture are placed in a tube carrier. The tube carrier containing the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture(s) must be placed in slot A of the sample drawer.

Depending on the number of samples to be processed, we recommend using 2 ml tubes (Sarstedt, cat. no. 72.693 or 72.694) or 14 ml 17 x 100 mm polystyrene, round-bottom tubes (Becton Dickinson, cat. no. 352051) for diluting the internal control, as described in the table on page 5. The volume can be split into 2 or more tubes.

Calculating the volume of internal control mixture

Tube type	Name on QIAsymphony touchscreen	Calculation of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture volume per tube
Microtube 2 ml with cap; microtube 2 ml, PP, SKIRTED, (Sarstedt, cat. No. 72.694)	SAR#72.694 T2.0 ScrewSkirt	(n x 120 μl) + 360 μl*
Microtube 2 ml with cap; microtube 2 ml, PP, NON-SKIRTED, (Sarstedt, cat. No. 72.693)	SAR#72.693 T2.0 Screw	(n x 120 μl) + 360 μl*
Tube 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051)	BD#352051 FalconPP 17x100	(n x 120 μl) + 600 μl†

* Use this equation to calculate the required volume of internal control mixture (n = number of samples; 120 μl = volume of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture; 360 μl = void volume required per tube). For example, for 12 samples (n = 12): (12 x 120 μl) + 360 μl = 1800 μl. Do not fill the tube with more than 1.9 ml (i.e., a maximum of 12 samples per tube). If more than 12 samples will be processed, use additional tubes, ensuring that the void volume is added per tube.

† Use this equation to calculate the required volume of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture (n = number of samples; 120 μl = volume of internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture; 600 μl = void volume required per tube). For example, for 96 samples (n = 96): (96 x 120 μl) + 600 μl = 12,120 μl.

See www.qiagen.com/goto/dsphandbooks for required inserts.

Using FIX labware

Using liquid-level detection (LLD) for sample transfer allows the use of primary and secondary tubes. However, this requires certain dead volumes in the respective tubes. In order to minimize dead volumes, secondary tubes should be used without liquid-level detection. Specific FIX labware is available (e.g., SAR_FIX_#72.694 T2.0 ScrewSkirt) which can also be selected on the touchscreen of the QIAsymphony SP. This tube/rack type imposes aspiration restrictions. The sample is aspirated at a particular height in the tube that is defined by the volume of sample to be transferred. Therefore, it is essential to make sure that the volume listed in the labware list is used. Labware lists are available for download from www.qiagen.com/goto/dsphandbooks.

Sample tubes that can be used with or without liquid-level detection and required sample volumes are listed at www.qiagen.com/goto/dsphandbooks. Do not use volumes greater or lower than the required volume since this may lead to errors during sample preparation.

Tubes for use with liquid-level detection and tubes that are not for liquid-level detection can be processed within one batch/run.

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.

Plasma, serum, and CSF samples

The purification procedure is optimized for use with plasma, serum, or CSF samples. Blood samples treated with EDTA or citrate as anticoagulant can be used for plasma preparation. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once. After collection and centrifugation, plasma, serum, or CSF can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –20°C or –80°C. Frozen plasma or serum must not be thawed more than once. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in a potential reduction in viral titers and, therefore, reduced yields of viral nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x *g* for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately. Centrifugation at low *g*-forces does not reduce viral titers.

Limitations

Blood samples treated with serum clot activator may cause reduced yields of viral nucleic acids. Do not use Greiner Bio-One® VACUETTE® Blood Collection Tubes containing Z Serum Clot Activator.

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