April 2021

QIAsymphony® SP Protocol Sheet

Bisulfite140_HC_V1

This document is the Bisulfite140_HC_V1 *QIAsymphony SP Protocol Sheet*, R1, for QIAsymphony Bisulfite® Kit.

QIAGEN

General information

The QIAsymphony Bisulfite Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Because the type of samples that can be processed using the QIAsymphony Bisulfite Kit can vary greatly, there is also a variety of different pretreatments, optimized for specific sample types.

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

Bisulfite140_HC_V1

Kit	QIAsymphony Bisulfite Kit
Sample material	Isolated DNA from different sources
Protocol name	Bisulfite140_HC_V1
Default Assay Control Set	ACS_ Bisulfite140_HC_V1
Elution Volume	40 µl, 50 µl, 60 µl, 70 µl, 80 µl, 90 µl
Elution solution	Buffer ATE
Required software version	Version 5.0 or higher

Materials required but not provided

For all sample types

- Reaction tubes (0.2 ml) or 8-well strips
- Thermal cycler with heated lid (because the bisulfite reaction is not overlaid with mineral oil, only thermal cyclers with heated lids are suitable for this procedure)
- Vortexer
- Thermomixer or shaker–incubator
- Microcentrifuge
- TopElute Fluid (60 ml) (cat. no. 1055628)

"Sample" drawer

Sample type	Bisulfite reactions from high content DNAs (2 µg – 100 ng)
Sample volume	140 µl
Primary sample tubes	See **www.qiagen.com/QIAsymphony-Bisulfite-Kits** for more information
Secondary sample tubes	See **www.qiagen.com/QIAsymphony-Bisulfite-Kits** for more information
Inserts	See **www.qiagen.com/QIAsymphony-Bisulfite-Kits** for more information
Other	n/a

n/a = not applicable.

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge (RC)
Position B1	TopElute
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/QIAsymphony-Bisulfite-Kits** for more information

Required plasticware

	One batch, 24 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	7	7
Disposable filter-tips, 1500 µl†‡	96	363
Sample prep cartridges§	21	84
8-Rod Covers¶	3	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 RC.
§ 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.

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 material safety data sheets (MSDSs), available from the product supplier.

Protocol: Bisulfite Conversion of Unmethylated Cytosines in low concentrated DNA

This protocol enables bisulfite conversion of DNA amounts of 1 ng – 2 µg in a volume of up to 20 µl (high concentration). For the bisulfite conversion of DNA amounts of 1 ng – 500 ng in a volume of up to 40 µl (low concentration) please go to Protocol Sheet LC 140.

The fully automated procedure processes a bisulfite reaction volume of 140 µl. DNA is eluted in 40–90 µl of Buffer ATE.

Important points before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in the step indicated in the respective pretreatment protocol.
- DNA protect buffer should turn from green to blue after addition to the DNA–bisulfite solution mixture, indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- Perform all centrifugation steps at room temperature (15–25°C).
- Before beginning the procedure, read "Important Notes", page 20 of the *QIAsymphony Bisulfite Handbook*.

Things to do before starting

- **Optional**: Set a thermomixer, heating block, or heated orbital incubator to 60°C to dissolve the bisulfite solution.
- The tubes containing the Buffer BD must be opened in the enzyme rack which is placed in the RC. It is recommended to only open the needed amount of tubes.
- Bisulfite protocols require TopElute Fluid (TOPE). Place an opened 60 ml bottle containing TOPE into the "Reagents and Consumables" drawer.
- For information about sample tubes compatible with a certain protocol, see the corresponding labware list (available at **www.qiagen.com/QIAsymphony-Bisulfite-Kits**).
- 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/QIAsymphony-Bisulfite-Kits**). This information also indicates which tubes can be used for different protocols.

- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 min before first use.
- Before loading the RC, remove the cover from the trough containing the magnetic particles and open the carrier RNA tubes. Make sure that the piercing lid is placed on the RC or, if using a partially used RC, make sure the Reuse Seal Strips have been removed.
- If samples are barcoded, orient samples in the tube carrier so that the barcodes face the barcode reader at the left side of the QIAsymphony SP.

Procedure

1. Thaw DNA to be used in the bisulfite reactions. Ensure that the bisulfite solution is completely dissolved.

   Note: If necessary, heat the bisulfite solution to 60°C and vortex until all precipitates are dissolved again.

   Note: Do not place dissolved bisulfite solution on ice.

2. Prepare the bisulfite reactions in 200 µl PCR tubes (not provided) according to Table 1. Add each component in the order listed.

   Note: The combined volume of DNA and RNase-free water must total 20 µl for high-concentration samples.

Table 1. Bisulfite reaction components

Component	High-concentration samples (1 ng – 2µg) Volume per reaction (µl)
DNA	Variable* (maximum 20 µl)
RNase-free water	Variable*
Bisulfite solution	85
DNA protect buffer	35
Total volume	**140**

* The combined volume of DNA and RNase-free water must total 20 µl.

3. Close the PCR tubes and mix the bisulfite reactions thoroughly. Store the tubes at room temperature (15–25°C).

   Note: The DNA protect buffer should turn from green to blue after addition to the DNA–bisulfite solution mixture, indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

4. Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 2.

   The complete cycle should take approximately 30 min.

   Optional: In some cases, it may be necessary to extend the 60°C cycle time up to 20 min to achieve complete bisulfite DNA conversion.

   Note: If using a thermal cycler that does not allow you to enter the reaction volume (140 µl), set the instrument to the largest volume setting available.

Table 2. Bisulfite conversion thermal cycler conditions

Step	Time	Temperature
Denaturation	5 min	95°C
Incubation	10 min*	60°C
Denaturation	5 min	95°C
Incubation	10 min*	60°C
Hold	Indefinite†	20°C

* In some cases, it may be necessary to extend the 60°C cycle time up to 20 min to achieve complete bisulfite DNA conversion.

† Converted DNA can be left in the thermal cycler overnight without any loss of performance.

5. Place the PCR tubes containing the bisulfite reactions into the thermal cycler. Start the thermal cycling incubation.

   Important: Since the bisulfite reaction is not overlaid with mineral oil, only thermal cyclers with heated lids are suitable for this procedure. It is important to use PCR tubes that close tightly.

   Converted DNA can be left in the thermal cycler overnight without any loss of performance.

6. Continue with the protocol "Bisulfite Conversion of Unmethylated Cytosines in Different Sample Types" (page 28 in the *QIAsymphony Bisulfite Handbook*).

Revision history

Date	Changes
R1, April 2021	Initial release

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 **http://www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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