April 2021

QIAsymphony® SP Protocol Sheet

Bisulfite140_LC_V1

This document is the Bisulfite140_LC_V1 *QIAsymphony SP Protocol Sheet*, R1, for QIAsymphony Bisulfite Kit.

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.

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

Note: For the Bisulfite140_LC_V1 protocol, samples may be lysed under denaturing conditions in the presence of Proteinase K and Buffer FTB.

Bisulfite140_LC_V1

Kit	QIAsymphony Bisulfite Kit, QIAsymphony Bisulfite FFPE Kit, QIAsymphony Bisulfite LyseAll Kit
Sample material	DNA isolated from different sources such as FFPE tissues, cell culture, whole blood, and tissues
Protocol name	Bisulfite140_LC_V1
Default Assay Control Set	ACS_ Bisulfite140_LC_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

Protocol overview

These protocols are for purification of total DNA from different kinds of samples using the QIAsymphony SP and the QIAsymphony Bisulfite, QIAsymphony Bisulfite FFPE, or QIAsymphony Bisulfite LyseAll Kit.

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

Sample type	Kit	Protocol	Elution volume (µl)
DNA (low input) 1–100 ng in a maximum volume of 40 µl	QIAsymphony Bisulfite Kit 931106	Bisulfite Conversion of Unmethylated Cytosines in Low Concentrated DNA; page 6	40, 50,60, 70, 80, 90
FFPE samples (10 µm slice)	QIAsymphony Bisulfite FFPE Kit 931206	Sample Lysis and Bisulfite Conversion of Unmethylated Cytosines in DNA Prepared from FFPE Tissue Samples; page 10	40, 50,60, 70, 80, 90
Cell culture, whole blood, tissues (0.5–20 µl blood, or 10–10^5 cells)	QIAsymphony Bisulfite LyseAll Kit 931306	Sample Lysis and Bisulfite Conversion of Unmethylated Cytosines in DNA Prepared from Whole Blood, Cultured Cells, or Tissue; page 13	40, 50,60, 70, 80, 90

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 form low content DNAs (1-500ng **in a maximum volume of 40 µl**), FFPE sample, whole blood, cell culture samples, tissue samples
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†‡	100	379
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 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.

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.

Protocol: Bisulfite Conversion of Unmethylated Cytosines in Low Concentrated DNA

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

The fully automated procedure processes a bisulfite reaction volume of 140 µl. DNA is eluted in 30–80 µl of Buffer ATE. Carrier RNA is added to the samples during the automated procedure to maximize yields from very small samples.

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 Kit Handbook*.

Things to do before starting

- **Optional**: Set a thermomixer, heating block, or heated orbital incubator to 60°C to dissolve the bisulfite solution.
- Dissolve the lyophilized carrier RNA in 1.6 ml Buffer ATE (provided in the QIAsymphony Bisulfite Kit) before using the kit for the first time. Transfer 400 µl to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. Add additional 1.2 ml Buffer ATE to each tube and mix by pipetting up and down several times.

  Note: It is important that the final volume of carrier RNA in the tubes of the enzyme rack is exactly 1.6 ml. Dissolved carrier RNA is stable for 4 weeks when stored at 2–8°C. For longer periods, store carrier RNA at –20°C.

  Note: For the inventory scan to be completed successfully, tubes containing carrier RNA must be opened and placed in the enzyme rack which is placed in the reagent cartridge. The carrier RNA, however, will not be used for reference protocols.

- The tubes containing the Buffer BD must be opened and placed in the enzyme rack which is placed in the reagent cartridge. 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 reagent cartridge, remove the cover from the trough containing the magnetic particles and open the carrier RNA and the BD Solution tubes. Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, 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 40 µl for low-concentration samples.

Table 1. Bisulfite reaction components

Component	High-concentration samples (1–500 ng) Volume per reaction (µl)
DNA	Variable* (maximum 40µl)
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 40 µl.

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

   Note: 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 Kit Handbook*).

Protocol: Sample Lysis and Bisulfite Conversion of Unmethylated Cytosines in DNA Prepared from FFPE Tissue Samples

This protocol is designed to be used with the QIAsymphony Bisulfite FFPE Kit for processing DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples.

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.
- If using FFPE samples on slides, scrape the FFPE slice from the slide and proceed with step 1.
- Deparaffinization solution solidifies at temperatures below 18°C. Incubate at 30°C to resolve.
- Precipitates may form in Lysis Buffer FTB. Make sure all precipitates are dissolved at 30°C.
- 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 Kit Handbook*.

Things to do before starting

- Dissolve the lyophilized carrier RNA in 1.6 ml Buffer ATE (provided in the QIAsymphony Bisulfite Kit) before using the kit for the first time. Transfer 400 µl to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. Add additional 1.2 ml Buffer ATE to each tube and mix by pipetting up and down several times.

  Note: It is important that the final volume of carrier RNA in the tubes of the enzyme rack is exactly 1.6 ml. Dissolved carrier RNA is stable for 4 weeks when stored at 2–8°C. For longer periods, store carrier RNA at –20°C.

  Note: For the inventory scan to be completed successfully, tubes containing carrier RNA must be opened and placed in the enzyme rack which is placed in the reagent cartridge. The carrier RNA, however, will not be used for reference protocols.
- Equilibrate samples and buffers to room temperature.

- **Optional**: If performing the deparaffinization, lysis, and decrosslinking of the FFPE slice in a 1.5 ml tube (see step 1), set a heating block to 56°C.
- **Optional**: Set a thermomixer, heating block, or heated orbital incubator to 60°C to dissolve the bisulfite solution.
- Bisulfite protocols require 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 reagent cartridge, 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 reagent cartridge or, if using a partially used reagent cartridge, 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

Deparaffinization, lysis, and decrosslinking of FFPE slice

1. Place a FFPE slice (10 µm) in a in a 1.5 ml tube and add 150 µl deparaffinization solution.
2. Flick or vortex the tube until all paraffin is dissolved.

   Note: The deparaffinization solution will form a layer.
3. Add 20 µl distilled water, 15 µl Lysis Buffer FTB, and 5 µl Proteinase K.

   Note: A master mix comprising distilled water, Lysis Buffer FTB, and Proteinase K may be prepared in advance.
4. Vortex and briefly centrifuge the samples.
5. Perform the lysis and decrosslinking **Error! Reference source not found.**in a thermal block. Incubate the tubes in a thermal block set to 56°C for 30 min to lyse the tissues. Ensure that tissues are completely lysed (the solution will become homogeneous); if not, incubate the tubes for an additional 30 min at 56°C. Once all tissues are lysed, increase the temperature of the heating block to 95°C for 60 min for the decrosslinking step.

6. Place the PCR tubes containing the lysis reactions into the thermal cycler. Start the thermal cycling incubation.

   Note: Samples should be kept at room temperature (15–25°C). Proceed as soon as possible with bisulfite conversion.

Bisulfite conversion of DNA

1. Make sure 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. Remove the deparaffinization solution (approx. 130 µl) from the lysis reactions.

   Remove as much deparaffinization solution as possible without disturbing the lysed sample material to make sufficient space in the reaction tube for the bisulfite reaction components (Table 3).

   Note: Small amounts of remaining deparaffinization solution have no effect on the bisulfite reaction.

3. Prepare the bisulfite reactions by adding the reagents listed in Table 3. Add each component in the order listed.

   Optional: If using 1.5 ml tubes for the deparaffinization, lysis, and decrosslinking, transfer the lysis reactions remaining from step 8 to 200 µl tubes. Add the reagents to the 200 µl tubes in the order listed in Table 3.

Table 3. Bisulfite reaction components

Component	Volume per reaction (µl)
Lysis reaction	Approx. 40
Bisulfite Solution	85
DNA Protect Buffer	15
Total volume	**140**

4. Proceed to "Protocol: Bisulfite Conversion of Unmethylated Cytosines in Low Concentrated DNA", starting with step 3, to perform the bisulfite DNA conversion.

Protocol: Sample Lysis and Bisulfite Conversion of Unmethylated Cytosines in DNA Prepared from Whole Blood, Cultured Cells, or Tissue

This protocol is designed to be used with the QIAsymphony LyseAll Kit for processing DNA from whole blood, cultured cells, or tissue samples.

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.
- Precipitates may form in Lysis Buffer FTB. Make sure all precipitates are dissolved at 30°C.
- 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 Kit Handbook*.

Things to do before starting

- Dissolve the lyophilized carrier RNA in 1.6 ml Buffer ATE (provided in the QIAsymphony Bisulfite Kit) before using the kit for the first time. Transfer 400 µl to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. Add additional 1.2 ml Buffer ATE to each tube and mix by pipetting up and down several times.

  Note: It is important that the final volume of carrier RNA in the tubes of the enzyme rack is exactly 1.6 ml. Dissolved carrier RNA is stable for 4 weeks when stored at 2–8°C. For longer periods, store carrier RNA at –20°C.

  Note: For the inventory scan to be completed successfully, tubes containing carrier RNA must be opened and placed in the enzyme rack which is placed in the reagent cartridge. The carrier RNA, however, will not be used for reference protocols.
- Equilibrate samples and buffers to room temperature.
- **Optional**: Set a thermomixer, heating block, or heated orbital incubator to 60°C to dissolve the bisulfite solution.
- Bisulfite protocols require 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 reagent cartridge 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 reagent cartridge or, if using a partially used reagent cartridge, 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

Sample lysis

Note: If working with whole blood samples, follow steps 1–10. If working with cultured cells, follow steps 11–16. If working with tissue samples, follow steps 17–21.

Whole blood samples

1. Dilute the blood sample (maximum 20 µl) 1:20 with Buffer EL (e.g., add 19 µl Buffer EL to 1 µl blood).
2. Incubate at room temperature (15–25°C) for 10–15 min.

   Note: Invert tubes several times during incubation.
3. Centrifuge at maximum speed for 5 min.
4. Discard supernatant and add an additional 125 µl Buffer EL.

   Important: Make sure to not disturb the blood cell pellet while removing supernatant.
5. Centrifuge at maximum speed for 1 min.
6. Resuspend pellet in 10 µl PBS and transfer into a 200 µl reaction tube or into 8-well strips (not provided).

   Important: Do not place cells on ice, as this will cause Lysis Buffer FTB to precipitate.

7. Add 10 µl distilled water, 15 µl Lysis Buffer FTB, and 5 µl Proteinase K.

   Note: A master mix comprising of distilled water, Lysis Buffer FTB, and Proteinase K may be prepared in advance.

8. Vortex and briefly centrifuge the samples.
9. Incubate samples for 30 min at 56°C.
10. Proceed as soon as possible with Bisulfite conversion, step 23.

    Note: Samples should be kept at room temperature.

Cultured cells

11. Harvest cells according to your current protocol.
12. Resuspend cells in 10 µl PBS.

    Note: Do not use more than 1 x 105 cells per 10 µl PBS.

    Important: Do not place cells on ice, as this will cause Lysis Buffer FTB to precipitate.

13. Add 10 µl distilled water, 15 µl Lysis Buffer FTB, and 5 µl Proteinase K.

    Note: A master mix comprising distilled water, Lysis Buffer FTB, and Proteinase K may be prepared in advance.

14. Vortex and briefly centrifuge the samples.
15. Incubate samples for 30 min at 56°C.
16. Proceed as soon as possible with bisulfite conversion, step 23.

    Note: Samples should be kept at room temperature (15–25°C).

Tissue samples

17. Add 20 µl distilled water to the tissue sample.

    Note: Do not use more than 100 µg tissue per 20 µl distilled water.

    Important: Do not place tissues on ice, as this will cause Lysis Buffer FTB to precipitate.

18. Add 15 µl Lysis Buffer FTB and 5 µl Proteinase K.

    Note: A master mix comprising Lysis Buffer FTB and Proteinase K may be prepared in advance.

19. Vortex and briefly centrifuge the samples.
20. Incubate samples for 30 min at 56°C.
21. Proceed as soon as possible with bisulfite conversion, step 23.

    Note: Samples should be kept at room temperature (15–25°C).

Bisulfite conversion

22. 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.

23. Prepare the bisulfite reactions in 200 µl PCR tubes (not provided) according to Table 3. Bisulfite reaction components. Add each component in the order listed.

24. Proceed to Protocol "Protocol: Bisulfite Conversion of Unmethylated Cytosines in Low Concentrated DNA", starting with Step 3, to perform the bisulfite DNA conversion.

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

Notes

Notes

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