June 2021

QIAsymphony® Bisulfite Kit Handbook

For sample lysis and complete bisulfite conversion/cleanup of DNA from FFPE, blood, cultured cells, or tissue samples, optimized for methylation analysis using the QIAsymphony SP

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAsymphony Bisulfite Kit **Catalog no.** **No. of reactions**	**(192)*** **931106** **192**
Bisulfite Solution	12 x 1.5 ml
DNA Protect Buffer	4 x 1.9 ml
Reagent Cartridge†‡	2
Enzyme Rack	2
Piercing Lid	2
Buffer ATE‡	20 ml
Carrier RNA	310 µg
Reuse Seal Set§	2

* For 192 x 140 µl preps.

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 9 for Safety Information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

QIAsymphony Bisulfite FFPE Kit Catalog no. No. of reactions	(192)* 931206 192
EpiTect® Fast FFPE Lysis Kit	
Deparaffinization Solution	4 x 8 ml
Lysis Buffer FTB	4 x 0.8 ml
Proteinase K	4 x 0.65 ml
QIAsymphony Bisulfite Kit	
Bisulfite Solution	12 x 1.5 ml
DNA Protect Buffer	4 x 1.9 ml
Reagent Cartridge†‡	2
Enzyme Rack	2
Piercing Lid	2
Buffer ATE‡	20 ml
Carrier RNA	310 µg
Reuse Seal Set§	2

* For 192 x 140 µl preps.

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 9 for Safety Information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

QIAsymphony Bisulfite LyseAll Kit Catalog no. No. of reactions	(192)* 931306 192
EpiTect Fast LyseAll Lysis Kit	
Buffer EL	4 x 25 ml
Lysis Buffer FTB	4 x 0.8 ml
Proteinase K	4 x 0.65 ml
QIAsymphony Bisulfite Kit	
Bisulfite Solution	12 x 1.5 ml
DNA Protect Buffer	4 x 1.9 ml
Reagent Cartridge†‡	2
Enzyme Rack	2
Piercing Lid	2
Buffer ATE‡	20 ml
Carrier RNA	310 µg
Reuse Seal Set§	2

* For 192 x 140 µl preps.

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 9 for Safety Information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

Shipping and Storage

The QIAsymphony Bisulfite, QIAsymphony Bisulfite FFPE, and QIAsymphony Bisulfite LyseAll Kits are shipped at room temperature (15–25°C). The reagent cartridges (RCs) should be stored at room temperature (15–25°C). Do not store the RCs at temperatures below 15°C.

Upon arrival, DNA Protect Buffer and the enzyme rack containing the Buffer BD should be stored at 2–8°C. However, short-term storage (up to 4 weeks) at room temperature does not affect their performance. The QIAsymphony Bisulfite FFPE and QIAsymphony Bisulfite LyseAll Kits contain ready-to-use Proteinase K solution that can be stored at room temperature. All other buffers including the Bisulfite Solution should be stored at room temperature and are stable for at least 6 months under these conditions.

Lyophilized carrier RNA can be stored at room temperature for 1 year. Carrier RNA should only be dissolved in Buffer ATE. Unused portions of carrier RNA dissolved in Buffer ATE should be frozen in aliquots at –30 to –15°C and can be stored for up to 1 year.

When stored correctly, the QIAsymphony Bisulfite, QIAsymphony Bisulfite FFPE, and QIAsymphony Bisulfite LyseAll Kits are stable until the expiration date on the kit box.

Partially used RCs can be stored for a maximum of 4 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If an RC is partially used, replace the cover of the trough containing the magnetic particles, seal the buffer troughs with the provided Reuse Seal Strips, and close the Buffer BD concentrate and the carrier RNA tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

To avoid evaporation, the RC should be open for a maximum of 25 hours (including run times) at a maximum environmental temperature of 30°C.

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

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

Intended Use

The QIAsymphony Bisulfite, QIAsymphony Bisulfite FFPE, and QIAsymphony Bisulfite LyseAll Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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.

CAUTION 	DO NOT add bleach or acidic solutions directly to the Bisulfite (W2) Solution or to waste containing Buffer QSB1.

Buffer QSB1 contains a guanidine salt, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilled, clean with suitable laboratory detergent and water.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony Bisulfite, QIAsymphony Bisulfite FFPE, and QIAsymphony Bisulfite LyseAll Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Epigenetics describes the study of heritable changes in gene function that occur without a change in the nuclear DNA sequence. In addition to RNA-associated silencing and histone modification, a major epigenetic mechanism in higher-order eukaryotes is DNA methylation.

Methylation of DNA occurs on cytosine residues, especially on CpG dinucleotides enriched in small regions of DNA (<500 bp). These regions, with a GC content greater than 55%, are known as CpG islands. They are usually clustered around the regulatory region of genes and can affect the transcriptional regulation of these genes. Methylation of CpG islands by DNA methylases has been shown to be associated with gene inactivation and plays an important role in the development of cancer and cell aging. Reversal of DNA methylation at these sites is a potential therapeutic strategy as this reversal may restore expression of transcriptionally silenced genes. In addition to CpG, methylated cytosine residues are also found at CpNpG or CpNpN sites (N = A, T, or C) in plants.

The methylation status of a DNA sequence can best be determined using bisulfite conversion. Bisulfite treatment of the target DNA results in conversion of unmethylated cytosine residues into uracil, leaving the methylated cytosines unchanged. Therefore, bisulfite treatment gives rise to different DNA sequences for methylated and unmethylated DNA (see below).

	Original sequence	**After bisulfite sequence**
Unmethylated DNA	N-C-G-N-C-G-N-C-G-N	N-U-G-N-U-G-N-U-G-N
Methylated DNA	N-C-G-N-C-G-N-C-G-N	N-C-G-N-C-G-N-C-G-N

The most critical step for correct determination of methylation pattern is the complete conversion of unmethylated cytosines. This is achieved by incubating the DNA in high bisulfite salt concentrations at high temperature and low pH. These harsh conditions often lead to a high degree of DNA fragmentation and subsequent loss of DNA during purification. Purification is necessary to remove bisulfite salts and chemicals used in the conversion process that inhibit sequencing. Common bisulfite procedures usually require high amounts of input DNA to compensate for DNA degradation during conversion and DNA loss during purification that often lead to low DNA yield, highly fragmented DNA, and irreproducible conversion rates.

The QIAsymphony Bisulfite Kits now provide a very fast and streamlined procedure for efficient conversion and purification of DNA prepared from formalin-fixed, paraffin-embedded (FFPE), blood, cell, or tissue samples. The kits combine the well-known EpiTect Fast Bisulfite conversion chemistry with the proven, performance-leading magnetic-particle technology to provide high-quality DNA, which is suitable for direct use in downstream applications, such as Pyrosequencing®, PCR or NGS applications, or for storage for later use. The QIAsymphony Bisulfite Kits contain highly concentrated Bisulfite Solution, which reduces the time required to convert unmethylated cytosine residues into uracil in little as 30 minutes, as well as preparation buffers that make it unnecessary to isolate the DNA prior to bisulfite treatment. DNA fragmentation is prevented during the bisulfite conversion by the unique DNA Protect Buffer, which contains a pH-indicator dye as a mixing control in reaction setup, allowing confirmation of the correct pH for cytosine conversion.

Furthermore, the bisulfite thermal cycling program provides an optimized series of incubation steps necessary for thermal DNA denaturation and subsequent sulfonation and cytosine deamination, enabling high cytosine conversion rates of over 99%. Desulfonation, the final step in chemical conversion of cytosines, is achieved by a convenient integrated step included in the purification procedure.

Purified DNA is free of proteins, nucleases, and inhibitors. The QIAsymphony SP performs all steps of the sample extraction procedure after lysis according to the pretreatment protocols. Up to 96 samples are processed in a single run.

The final elution volume can be as low as 40 µl, though this may result in a yield reduction.

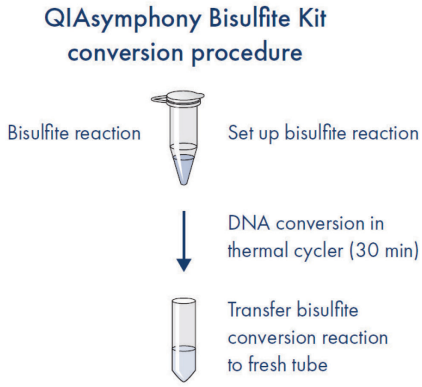
Principle and procedure

The QIAsymphony Bisulfite Kit procedure comprises a few simple steps: preparation of DNA from sample, bisulfite-mediated conversion of unmethylated cytosines, transfer of sample to the QIAsymphony SP module, automated binding of the converted single-stranded DNA to the magnetic beads, washing, desulfonation of beads-bound DNA, washing of the bead-bound DNA to remove the desulfonation agent, and elution of the pure, converted DNA from the beads. Sample preparation is different for FFPE slices, whole blood, and cell cultures or tissues, whereas the procedure for bisulfite conversion of extracted DNA is the same for all sample types (see the flowcharts on pages 13–15). The eluted, bisulfite-converted DNA is suited for all techniques currently used for the analysis of DNA methylation, including PCR, real-time PCR, methylation-specific PCR, bisulfite sequencing (direct and cloning), COBRA, Pyrosequencing, and NGS applications.

Bisulfite solution

The bisulfite solution is conveniently provided in separate aliquots that are ready to use. The bisulfite in each aliquot is supplied in a unique formulation that provides the optimal pH for complete conversion of cytosine to uracil, without the need for tedious pH adjustment. Bisulfite Solution can be stored at room temperature (15–25°C) for at least 6 months.

After setting up the bisulfite reaction, continue with the procedure shown in the flowchart QIAsymphony Bisulfite Conversion Procedure below.



DNA protect buffer

The DNA protect buffer is uniquely formulated to prevent the fragmentation usually associated with bisulfite treatment of DNA at high temperatures and low pH values. It also provides effective DNA denaturation, resulting in the single-stranded DNA necessary for complete cytosine conversion. In addition, the DNA protect buffer contains a pH indicator dye as a mixing control and to allow confirmation of the correct pH for cytosine conversion.

Bisulfite thermal cycling

The thermal cycling program provides an optimized series of short incubation steps necessary for thermal DNA denaturation and subsequent sulfonation and cytosine deamination, enabling the highest cytosine conversion rates.

QIAsymphony technology

The QIAsymphony technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (Figure 1). The purification procedure is designed to ensure safe and reproducible handling of precious or potentially infectious samples, and comprises 5 steps: lyse, bind, desulfonation, wash, and elute (see the flowchart next page). The user can choose different elution volumes between 40 and 90 µl of modified TE Buffer (Buffer ATE). DNA yields depend on sample type, age, and storage.

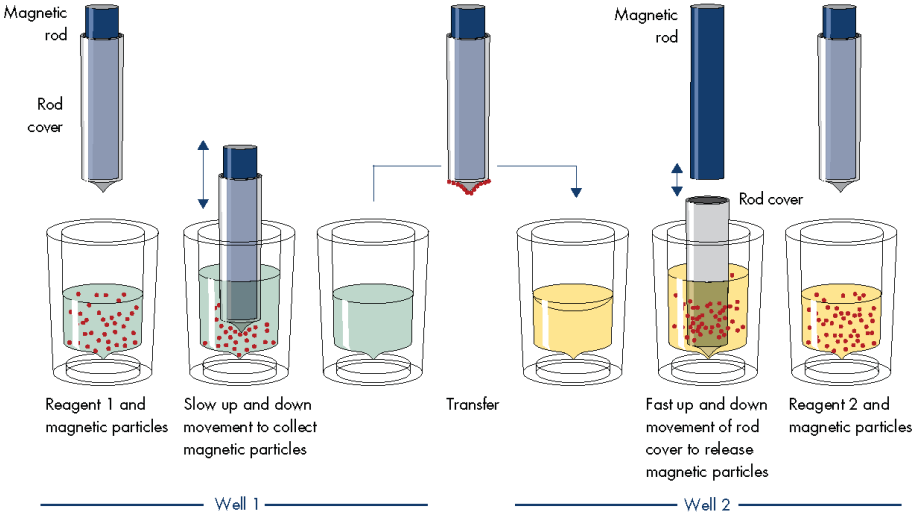
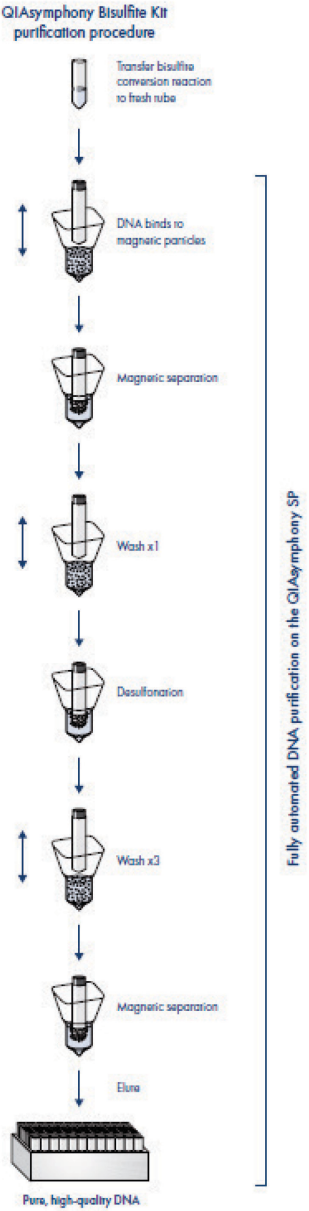


Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.


QIAsymphony Bisulfite Kit
purification procedure
Transfer bisulfite
conversion reaction
to fresh tube
DNA binds to
magnetic particles
Magnetic separation
Wash x1
Desulfonation
Wash x3
Magnetic separation
Elute
Pure, high-quality DNA
Fully automated DNA purification on the QIAsymphony SP

Carrier RNA

The kit is supplied with lyophilized carrier RNA to be used in the QIAsymphony Bisulfite Low Content protocols. Carrier RNA enhances binding of DNA to the magnetic particles, especially if there are very few target molecules in the sample. The concentration of carrier RNA allows the procedure to be used as a generic purification system that is compatible with many different amplification systems. Note that eluates contain both carrier RNA and DNA from the sample, with the amount of carrier RNA greatly exceeding the amount of DNA. For details about using carrier RNA, refer to the "Things to do before starting" section in the DNA purification protocols.

Optimized buffers

The QIAsymphony Bisulfite Kit contains carefully optimized buffers, enabling effective lysis of samples (FFPE, cells, tissue, and whole blood) in DNA isolation procedures, deparaffinization of FFPE tissues, and maximum cytosine conversion from the EpiTect Fast DNA Conversion Kits. After efficient binding to the magnetic beads, all the subsequent steps are performed automatically by the QIAsymphony. The bead-bound DNA is washed, which efficiently removes residual bisulfite solution. After desulfonation, the DNA is further desalted before elution from the magnetic beads using Buffer ATE.

Storage stability of converted and purified DNA

The DNA converted and purified using QIAsymphony Bisulfite Kits can be stored at –20°C.

Description of protocols

There are two different kinds of DNA purification protocols, which can be used in conjunction with the pretreatment protocols and the different kits (see protocol sheets at **www.qiagen.com/QIAsymphony-Bisulfite-Kits**). The choice of kit and the corresponding protocol to use is determined by the type of starting material.

- The Bisulfite 140 High Content protocol starts the conversion reaction from a high quality DNA sample with a high concentration.
- The Bisulfite 140 Low Content protocol starts the conversion reaction from a high quality DNA sample with a lower concentration or with a pretreatment of FFPE, cell culture, whole blood, or tissue samples. This protocol uses carrier RNA to improve the yield.
- Both protocols are optimized for elution in small volumes (40–90 µl). The protocols use TopElute Fluid (TOPE) to overlay eluates during the elution process. TOPE is not transferred to the elution labware.

FFPE tissues are processed with the QIAsymphony FFPE Bisulfite Kit. This kit consists of the EpiTect Fast FFPE Lysis Kit, containing specialized buffers for efficient deparaffinization and lysis of the tissue sample, and the QIAsymphony Bisulfite Kit for the bisulfite conversion of the extracted DNA.

Whole blood, cultured cells, or tissue samples are processed with the QIAsymphony Bisulfite LyseAll Kit. This kit consists of the EpiTect Fast LyseAll Lysis Kit, including an innovative lysis buffer, and the QIAsymphony Bisulfite Kit for the bisulfite conversion of the extracted DNA.

All protocols achieve the same cytosine conversion rates and lead to equal DNA recoveries after purification of converted DNA, independent of DNA starting amounts. The purified DNA is ready to use in downstream applications.

Equipment and Reagents Supplied by 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.

- 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
- Pipets and pipet tips (we recommend pipet tips with aerosol barriers for preventing cross-contamination)
- TopElute Fluid (60 ml) (cat. no. 1055628)
- Sample Prep Cartridges, 8-well cartridges (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 200 µl and 1500 µl (cat. nos. 990332 and 997024, respectively)
- Sample tubes or plates (e.g., 2 ml sample tubes with screw caps [Sarstedt®, cat. no. 72.693] or without caps [Sarstedt, cat. no. 72.608], or S-Blocks [QIAGEN, cat. no. 19585])

  Compatible primary and secondary tube formats are listed at **www.qiagen.com/QIAsymphony/Resources**
- Elution tubes. Compatible elution tube formats are listed at **www.qiagen.com/QIAsymphony/Resources**
- For additional materials required for specific sample preparations, please refer to the protocol sheets at **www.qiagen.com/QIAsymphony-Bisulfite-Kits**

Important Notes

Starting material, yield, and size of DNA

Bisulfite conversion also depends on the nature of DNA used as starting material. Genomic DNA should be used for bisulfite treatment without any previous restriction digest step. If working with plasmid DNA, the DNA should be linearized before starting the procedure due to the very quick reannealing of single-stranded DNA after the denaturation step.

The amount of starting material for use in QIAsymphony Bisulfite procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts is given in the individual protocols (see protocol sheets at **www.qiagen.com/QIAsymphony-Bisulfite-Kits**).

The QIAsymphony SP can process DNA input amounts ranging from 1 ng to 2 µg using the protocol for purified DNA. DNA can be prepared from FFPE tissue (10 µm in thickness). FFPE samples can be used directly with the QIAsymphony Bisulfite FFPE Kit and the corresponding protocol (Protocol Sheet). DNA can be prepared from 0.5 to 20 µl blood or 10–10^5 cells (as little as 60 pg of DNA). Blood, cultured cells, or tissue samples can be used directly with the QIAsymphony Bisulfite LyseAll Kit and the corresponding protocol (Protocol Sheet). Table 1 provide additional information about protocol options.

Table 1. Protocol description depending on the sample type used in the Bisulfite conversion reaction using the different QIAsymphony Busulfite Kits

Sample type	Kit	Protocol Sheet	Elution volume (µl)
DNA (high input) 1 ng – 2 µg in a volume of up to 20 µl	QIAsymphony Bisulfite Kit cat. no. 931106	Bisulfite 140 High Content	40, 50,60, 70, 80, 90
DNA (low input) 1–500 ng in a maximum volume of 40 µl	QIAsymphony Bisulfite Kit cat. no. 931106	Bisulfite 140 Low Content	40, 50, 60, 70, 80, 90
FFPE samples (10 µm slice)	QIAsymphony Bisulfite FFPE Kit cat. no. 931206	Bisulfite 140 Low Content	40, 50, 60, 70, 80, 90
Cell culture, whole blood, and tissues (0.5–20 µl blood or 10–10^5 cells)	QIAsymphony Bisulfite LyseAll Kit cat. no. 931306	Bisulfite 140 Low Content	40, 50, 60, 70, 80, 90

Note: Some loss in volume is expected in the eluate.

The yield of DNA purified after bisulfite conversion depends on the amount of DNA and source of the starting material.

The size of the template DNA can vary between 500 bp (in laser microdissections) and 30 kb (fresh samples or blood). DNA purified from serum, urine, or FFPE tissue may be <500 bp in length.

Note: The purified sample will contain considerably more carrier RNA than DNA. Carrier RNA does not influence downstream applications.

Lysis with Proteinase K

The QIAsymphony Bisulfite FFPE Kit and the QIAsymphony Bisulfite LyseAll Kit contain Proteinase K, which possesses a high specific activity that remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and is particularly suitable for short digestion times.

Use of TOPE

The bisulfite protocols are optimized for maximum recovery of low elution volumes. Bisulfite protocols eluting in small volumes of Buffer ATE (40–90 µl) use TOPE during the elution process.

Automated purification with the QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer before a run. Start the protocol and remove purified DNA from the "Eluate" drawer after processing. Refer to the user manual provided with the instrument for operating instructions.

Loading RCs into the "Reagents and Consumables" drawer

Reagents for purification of DNA are contained in an innovative RC (see Figure 2). Each trough of the RC contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used RCs can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

Figure 2. QIAsymphony RC. The RC contains all reagents required for the protocol run.

Before starting the procedure, ensure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the RC frame, vortex it vigorously for at least 3 minutes, and replace it in the RC frame before the first use. Place the RC into the RC holder. Place the enzyme rack with the diluted carrier RNA and BD Solution into the RC holder. Before using an RC for the first time, place the piercing lid on top of the RC (Figure 3, next page).

Important: The piercing lid is sharp. Take care when placing it onto the RC. Make sure to place the piercing lid onto the RC in the correct orientation.

After the magnetic-particle trough cover is removed and the carrier RNA tubes are opened (screw caps can be stored in dedicated slots, see Figure 2), the RC is subsequently loaded into the "Reagents and Consumables" drawer.

Figure 3. Easy worktable setup with RCs.

Partially used RCs can be stored until needed again, see “Shipping and Storage” section, page 7.

TopElute Fluid

The bisulfite protocols require TOPE. An opened 60 ml bottle containing TOPE is placed into the “Reagents and Consumables” drawer.

Loading plasticware into the “Reagents and Consumables” drawer

Sample prep cartridges, 8-Rod Covers, TopElute (both preracked in unit boxes), and disposable filter-tips (200 µl tips provided in blue racks, 1500 µl tips provided in gray racks) are loaded into the “Reagents and Consumables” drawer.

See protocol sheets at **www.qiagen.com/QIAsymphony-Bisulfite-Kits** for the consumables required for QIAsymphony Bisulfite protocols.

For plasticware Ordering Information, see page 37.

Note: Both types of tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks, unit boxes for sample prep cartridges, or 8-Rod Covers manually before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

Loading the "Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the user manual provided with the instrument.

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place.

Loading the "Eluate" drawer

Load the required elution rack into the "Eluate" drawer. Do not load a 96-well plate onto "Elution slot 4". If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter. As long-term storage of eluates in the "Eluate" drawer may lead to evaporation of eluates, we strongly recommend using the cooling position.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers.

Storage and quality of purified DNA

DNA eluted in Buffer ATE is immediately ready for use in amplification reactions or can be stored at 2–8°C, –20°C, or at –80°C.

QIAsymphony Bisulfite procedures yield DNA free of proteins, nucleases, and inhibitors.

Quantification of DNA

Depending on the sample type, the yields of DNA obtained in the purification procedure might be below 1 µg and therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA might contain much more carrier RNA than target nucleic acids. We recommend using quantitative amplification methods to determine yields.

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. To remove magnetic-particle carryover, see Appendix B, page Appendix B: Removing Magnetic-Particle Carryover36.

Note: For accurate quantification of DNA eluted in Buffer ATE by absorbance at 260 nm, we recommend diluting the sample in elution buffer (Buffer ATE). Dilution of the sample in water may lead to inaccurate values. The elution buffer has a high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed. We therefore strongly recommend using elution buffer as a blank. Extra elution buffer to blank the spectrophotometer is provided in a separate bottle with QIAsymphony Bisulfite Kits.

Protocol: Bisulfite Conversion of Unmethylated Cytosines in Different Sample Types

This protocol enables bisulfite conversion of purified DNA, FFPE samples, whole blood, cultured cells, or tissue as described in the pretreatment protocols (see the protocol sheets at **www.qiagen.com/QIAsymphony-Bisulfite-Kits**). The protocol further describes the procedure for setting up the QIAsymphony SP and starting a run. See Table 1 for a summary of protocol options.

Bisulfite High Content protocol is designed to ensure efficient bisulfite conversion from high concentrated samples starting from a purified DNA. The fully automated procedure processes a bisulfite reaction volume of 140 µl. DNA is eluted in 40–90 µl of Buffer ATE.

Bisulfite Low Content protocol is designed to ensure efficient bisulfite conversion from low concentrated samples starting from a purified DNA or from FFPE, cell culture, whole blood or tissue samples. The fully automated procedure processes a bisulfite reaction volume of 140 µl. DNA is eluted in 40–90 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

- 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.
- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the user manual provided with the instrument for operating instructions.
- Before beginning the procedure, read “Important Notes” starting on page 19.

- Try to avoid vigorous shaking of the RC; otherwise, foam may be generated, which can lead to liquid-level detection problems.
- Ensure you are familiar with the protocol sheet corresponding to the procedure you want to use (**www.qiagen.com/QIAsymphony-Bisulfite-Kits**).

Things to do before starting

- **Optional**: Set a thermomixer, heating block, or heated orbital incubator to 60°C to dissolve the bisulfite solution.
- For Bisulfite LC protocols only: 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 5, 6, and 7 of the enzyme rack on the RC. 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 place d in the RC. The carrier RNA, however, will not be used for reference protocols.
- 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.
- 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.

- All protocols require TOPE. Place an opened 60 ml bottle containing TOPE into the "Reagents and Consumables" drawer.
- 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. Once the bisulfite conversion is complete, briefly centrifuge the PCR tubes containing the bisulfite reactions, and then transfer the complete bisulfite reactions to a QIAsymphony compatible tube format like a 2 ml screw cap tubes.

2. Transfer of precipitates in the solution will not affect the performance or yield of the reaction.

3. Ensure that the QIAsymphony SP is switched on.

   The power switch is located at the bottom, left corner of the QIAsymphony SP.

4. Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.

5. Load the required RC(s) and consumables into the "Reagents and Consumables" drawer.

   The tubes containing the carrier RNA and the Buffer BD must be opened and placed in the enzyme rack which is placed in the RC.

6. Press the **R+C** button in the touchscreen to open the screen that shows the consumables status (**Consumables/8-Rod Covers/Tubes/Filter-Tips/Reagent Cartridges**). Press the **Scan Bottle** button to scan the barcode of the bottle of TOPE with the handheld barcode scanner. Press **OK**.

   Ensure that the bottle of TOPE is scanned, opened, and placed into the "Reagents and Consumables" drawer before starting the inventory scan. Otherwise, the inventory scan must be repeated after scanning, opening, and placing the bottle of TOPE into the "Reagents and Consumables" drawer.

7. Perform an inventory scan of the "Reagents and Consumables" drawer.

8. Load the required elution rack into the "Eluate" drawer.

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

   If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter.

9. Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.

10. Using the touchscreen, enter the required information for each batch of samples to be processed.

    Enter the following information:

    - Sample information (depending on sample racks used)
    - Protocol ("Assay Control Set") to be run
    - 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 processing.

    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 DNA from the "Eluate" drawer.

    The DNA is ready to use or can be stored at 2–8°C, –20°C, or –80°C.

    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 magnet and the eluates transferred to a clean tube (see Appendix B, page 36).

    If the eluate drawer is closed when a batch is running (e.g., if elution racks which contain the eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message appears during the scan and must be closed (by pressing the **Close** button) before the run can be restarted.

    Result files are generated for each elution plate.

13. If the RC(s) is only partially used, seal it with the provided Reuse Seal Strips and close the carrier RNA and the BD Solution tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

    Note: For more information about storage of partially used RCs, see "Shipping and Storage" section, page 7.

14. Discard used sample tubes, plates, and waste according to your local safety regulations.

    See page 9 for Safety Information.

15. Clean the QIAsymphony SP.

    Follow the maintenance instructions in the user manual provided with the instrument.

16. Close the workstation drawers, and switch off the QIAsymphony SP.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

	Comments and suggestions
General handling	
Error message displayed in the touch screen	If an error message is displayed in the touchscreen during a protocol run, refer to "Troubleshooting" section in the user manual provided with the instrument.
Precipitate in reagent trough of opened cartridge	
a) Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard RC. Make sure to seal buffer troughs of partially used RC when not being used for DNA purification.
b) Storage of RC	Storage of RC under 15°C may lead to the formation of precipitates. If necessary, remove the trough containing Buffer QSL3 from the RC and incubate for 30 min at 37°C with occasional shaking to dissolve the precipitate. If the RC is already pierced, make sure that the RC is reclosed with the Reuse Seal Set and incubate the complete RC for 30 min at 37°C with occasional shaking in a water bath.
Low DNA yield	
a) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
b) Clogging of pipet tip due to insoluble material	Solid sample material was not removed from the digested sample prior to starting the QIAsymphony DNA purification procedure.

	Comments and suggestions
DNA does not perform well in downstream applications	
a) Insufficient DNA used in downstream application	See "Low DNA yield" for possible reasons. If possible, increase the amount of eluate used in the reaction.
b) Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the reaction accordingly.
Low conversion rate	
a) Incorrect thermal cycling conditions used	Use the thermal cycling conditions given in the protocol sheets.
b) Poor DNA quality (i.e., protein contamination)	Check that the A_{260}/A_{280} ratio of the sample DNA is between 1.7 and 1.9.
c) Amount of DNA used outside recommended range	Increase or decrease the amount of starting DNA material to stay within the range of 1 ng to 2 µg for purified DNA. For FFPE samples, cells, tissue, or blood, please refer to the indicated sample amounts in Table 1.
d) DNA protect buffer not added	Upon addition of DNA protect buffer, the DNA–bisulfite solution mixture should turn from green to blue indicating sufficient mixing and the correct pH for DNA binding to the MinElute® DNA spin column. If this color change does not occur, repeat the reaction ensuring that DNA Protect Buffer has been added.
e) Bisulfite solution contains precipitates	Heat the bisulfite solution to 60°C and vortex until all precipitates are dissolved again.
f) Insufficient time for bisulfite DNA conversion	Extend thermal cycling step of 60°C from 10 min to 20 min.
Incomplete deparaffinization	
a) Insufficient time for deparaffinization	Vortex the sample in deparaffinization solution until paraffin is visibly dissolved.
b) Tissue slice not exposed to Deparaffinization Solution	Vortex and invert tube to ensure entire slice is uniformly exposed to deparaffinization solution.

	Comments and suggestions
Incomplete lysis	
a) FFPE tissue slice not deparaffinized	Ensure paraffin is completely dissolved before adding Lysis Buffer FTB and Proteinase K.
b) Sample not exposed to Lysis Buffer FTB and Proteinase K	Ensure tissue is covered by lysis reagents (e.g., tissue should not be stuck to the tube cap). It may be necessary to fold the tissue into the solution using a clean pipet tip.
c) Insufficient time for lysis	Incubate the sample at 56°C for an additional 30 min.
d) Lysis reaction prepared incorrectly	Make sure to add all necessary components of the lysis reaction, as described in the protocol sheets.
Poor results in downstream PCR applications	
a) Little or no PCR product, even in control reaction	If performing hot-start PCR, confirm that the initial enzyme activation step was performed. Ensure that all PCR components were added and that suitable cycling conditions were used.
b) Failure of conversion reaction	The starting DNA was not sufficiently pure. Ensure that only high-quality DNA is used for the conversion reaction. Ensure that all steps of the modification and cleanup protocol were followed. Sample DNA was degraded before modification reaction. Ensure that sample DNA is handled and stored correctly. PCR primers were not appropriate or incorrectly designed. Check primer design. Amount of template DNA used in PCR was insufficient. Increase amount of template DNA.
Unexpected findings in buffers	
a) Color of DNA protect buffer changes from light green to olive during storage	DNA protect buffer is stable at 2–8°C for 1 year, and a change in color within this time has no influence on performance.
b) Bisulfite solution contains precipitates	Heat the bisulfite solution to 60°C and vortex until all precipitates are dissolved again.

Appendix A: Working with DNA

General handling

Proper microbiological aseptic technique should always be used when working with small sample sizes. Hands and dust particles may carry bacteria and molds and are the most common sources of contamination. Always wear latex or vinyl gloves while handling reagents and samples to prevent contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the purification procedure. These tubes are generally DNase-free.

Appendix B: Removing Magnetic-Particle Carryover

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

Carryover of magnetic particles in the eluate may affect the absorbance at 260 nm (A_{260}) in a spectrophotometer. To remove particles, the tube containing the eluate should be applied first to a suitable magnetic separator and the eluate transferred to a clean tube:

- Apply the tube containing the nucleic acids to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) until the magnetic particles are separated. If eluate is in microplates, apply the microplate to a suitable magnetic separator (e.g., QIAGEN 96-Well Magnet Type A, cat. no. 36915) until the magnetic particles are separated.
- If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw an aliquot for quantification and dilute as necessary.
- Measure the absorbance at 320, 280, and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 and 280 nm to correct for the presence of magnetic particles.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony Bisulfite Kit (192)	For 192 x 140 µl preps.: Includes 2 RCs enzyme racks and accessories	931106
QIAsymphony Bisulfite FFPE Kit (192)	For 192 x 140 µl preps.: Includes pre-treatment reagents, 2 RCs enzyme racks and accessories	931206
QIAsymphony Bisulfite LyseAll Kit (192)	For 192 x 140 µl preps.: Includes pre-treatment reagents, 2 RCs enzyme racks and accessories	931306
Related products		
Accessory Trough (10)	Accessory troughs for use with the QIAsymphony SP	997012
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIAsymphony SP	997008
Sample Carrier, plate, Qsym	Plate carrier for sample input. For use with the QIAsymphony SP	9017660
Reuse Seal Set (20)	Reuse Seal Set for sealing QIAsymphony reagent cartridges	997006
Tube Insert, 11 mm, sample carrier, Qsym	Primary tube adapter (11 mm) for use with the QIAsymphony tube carrier	9241033
Tube Insert, 13 mm, sample carrier, Qsym	Primary tube adapter (13 mm) for use with the QIAsymphony tube carrier	9241034
Tube Insert, 2 ml, sample carrier, Qsym	Secondary tube adapter (for 2 ml screw-cap tubes) for use with the QIAsymphony tube carrier	9241032

Product	Contents	Cat. no.
Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym	Adapter for holding Snap-Cap tubes	9020731
Cooling Adapter, tubes, 2 ml, Qsym	Cooling adapter for 2 ml screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9018088
Cooling Adapter, EMT, Qsym	Cooling adapter for EMT racks. For use in the QIAsymphony "Eluate" drawer	9018086
Cooling Adapter, MTP, RB, Qsym	Cooling adapter for round bottom microtiter plates (MTP). For use in the QIAsymphony "Eluate" drawer	9018085
Cooling Adapter, PCR, Qsym	Cooling adapter for PCR plates. For use in the QIAsymphony "Eluate" drawer	9018087
Adapter, tubes, 2 ml, Qsym	Adapter for 2 ml screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9018577
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP	990332
Filter-Tips, 1500 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP	997024
Tip Disposable Bags (15)	Tip disposal bags for use with the QIAsymphony SP	9013395

Product	Contents	Cat. no.
TopElute Fluid (60 ml)	60 ml TopElute Fluid for elution process in casework HE protocols.	1055628
QIAGEN Proteinase K (2 ml)	For protease digestion during DNA and RNA preparation. Contents: 2 ml (>600 mAU/ml, solution)	19131
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585

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.

Document Revision History

Date	Changes
04/2021	Initial release
06/2021	Updated the URL where the protocol sheets can be found.

Notes

Notes

Limited License Agreement for QIAsymphony Bisulfite Kit

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