June 2015

EpiTect® 96 Bisulfite Handbook

For complete bisulfite conversion and cleanup of DNA for methylation analysis in 96-well format

epigenomics

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EpiTect 96 Bisulfite Kit	**(2)**
Catalog no.	**59110**
Number of preps	**192**
Chemical Module	
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RNase-Free Water	3 x 10 ml
EpiTect DNA Protect Buffer Reservoir	1
EpiTect Conversion Plates	2
EpiTect Cover Foils	2
Purification Module	
EpiTect 96 Plates	2
EpiTect Elution Plates	4
Buffer BL*	4 x 31 ml
Buffer BW (concentrate)	2 x 52 ml
Buffer BD (concentrate)	2 x 3 ml
Buffer EB	15 ml
Carrier RNA	1350 µg
S-Blocks	2
AirPore Tape Sheets	25
Tape Pads	25
Top Elute Fluid	2 x 1.4 ml
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See "Safety Information" (page 6).

Shipping and Storage

The EpiTect 96 Bisulfite Kit is shipped at room temperature (15–25°C). Upon arrival, the DNA Protect Buffer and the Buffer BD should be stored at 2–8°C. However, short-term storage (up to 4 weeks) at room temperature does not affect performance.

All other buffers and the Bisulfite Mix should be stored at room temperature and are stable for at least 6 months under these conditions.

Dissolved Bisulfite Mix can be stored at –30 to –15°C for up to 4 weeks.

Lyophilized carrier RNA can be stored at room temperature for 1 year. Carrier RNA can only be dissolved in RNase-free water. Dissolved carrier RNA should be immediately added to Buffer BL, as described in "Things to do before starting" in each protocol. This solution should be prepared fresh, and is stable at 2–8°C for up to 48 hours. Unused portions of carrier RNA dissolved in RNase-free water should be frozen in aliquots at –30 to –15°C and can be stored for up to 1 year.

Intended Use

The EpiTect 96 Bisulfite Kit is intended for molecular biology applications. This product is 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 material 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 waste containing Buffer BL.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the EpiTect 96 Bisulfite Kit 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 sodium bisulfite. Incubation of the target DNA with sodium bisulfite 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 treatment**
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 a 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 usually 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 procedures. Common bisulfite procedures usually require high amounts of input DNA. However, due to DNA degradation during conversion and DNA loss during purification, such procedures often lead to low DNA yield, highly fragmented DNA, and irreproducible conversion rates.

The EpiTect 96 Bisulfite Kit provides a fast and streamlined 6-hour procedure for efficient conversion and purification of as little as 1 ng DNA. DNA fragmentation is prevented during the bisulfite conversion reaction 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 on-column step included in the purification procedure.

Principle and procedure

The EpiTect 96 Bisulfite Kit procedure comprises a few simple steps: bisulfite-mediated conversion of unmethylated cytosines; binding of the converted single-stranded DNA to the membrane of an EpiTect 96 Plate; washing; desulfonation of membrane-bound DNA; washing of the membrane-bound DNA to remove desulfonation agent; and elution of the pure, converted DNA from the EpiTect 96 Plate. The eluted DNA is suited for all techniques currently used for the analysis of DNA methylation, including PCR, real-time PCR, MSP-PCR, bisulfite sequencing (direct and cloning), COBRA, and Pyrosequencing®.

Bisulfite Mix

The Bisulfite Mix is conveniently provided in two separate aliquots (96 conversion reactions per aliquot). The sodium bisulfite in each aliquot is supplied in a unique formulation that provides the optimum pH for complete conversion of cytosine to uracil, without the need for tedious pH adjustment. The Bisulfite Mix must be dissolved in 9 ml RNase-free water before use (see "Things to do before starting", page 14, 19, 24, 29, 34, 40, 46, or 52). Reconstituted Bisulfite Mix can be stored at –20°C for up to 4 weeks.

DNA Protect Buffer

DNA Protect Buffer is uniquely formulated to prevent the DNA 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, 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 incubation steps necessary for thermal DNA denaturation and subsequent sulfonation and cytosine deamination, enabling highest cytosine conversion rates.

Carrier RNA

Carrier RNA is provided to enhance the binding of small quantities of DNA to the EpiTect 96 Plate. It should be dissolved in RNase-free water before use (see "Things to do before starting", pages 14, 19, 24, 29, 34, 40, 46, and 52). If using more than 100 ng genomic DNA template, it is not necessary to use carrier RNA, though we strongly recommend its use when processing fragmented DNA or DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Carrier RNA does not influence downstream applications.

Optimized buffers

The EpiTect 96 Bisulfite Kit contains carefully optimized buffers enabling maximum cytosine conversion and subsequent DNA purification. Buffer BL promotes binding of the converted single-stranded DNA to the EpiTect 96 Plate. Subsequently, the membrane-bound DNA is washed using Buffer BW, which efficiently removes residual sodium bisulfite. After desulfonation using Buffer BD, the DNA is further desalted using Buffer BW. Finally, the EpiTect 96 Plate is washed with ethanol before the converted DNA is eluted using Buffer EB.

Top Elute Fluid

Top Elute Fluid is used in the elution step of the vacuum protocol to increase the vacuum pressure on each sample. In addition, it helps provide even pressure across the whole 96-well plate. Top Elute Fluid is chemically inert and does not influence any downstream applications.

Storage stability of converted and purified DNA

DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored at –20°C for at least 3 years without decrease in quality or conversion.

Description of protocols

The EpiTect 96 Bisulfite Kit is suited for a wide range of DNA starting amounts. The standard protocol "Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA using a Centrifuge" can be performed using a centrifuge (page 14) or a vacuum manifold (page 34) and allows conversion of 1 ng – 2 µg DNA.

To efficiently convert very small amounts of DNA from limited and/or precious samples, such as low concentrations of DNA in solution from microdissected biopsies or freely circulating DNA, use the protocol "Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA from Low-Concentration Solutions using a Centrifuge". This protocol can be performed using a centrifuge (page 19) or a vacuum manifold (page 40) and optimizes the conversion of 1–500 ng DNA in a volume of up to 40 µl.

The protocol "Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA Isolated from FFPE Tissue Samples using a Centrifuge" is designed for conversion of DNA from formalin-fixed, paraffin-embedded (FFPE) tissues and includes an optimized step to facilitate binding of DNA. This protocol can be used with 1 ng – 2 µg DNA. Both centrifugation (page 24) and vacuum (page 46) protocols are available.

The protocol "Sodium Bisulfite Conversation of Unmethylated Cytosines in Small Amounts of Fragmented DNA using a Centrifuge" is intended for the conversion of small amounts of DNA that might also be fragmented. With an optimized step to facilitate binding of DNA, this protocol can be used with less than 500 pg DNA in a volume of up to 40 µl and can be performed on a centrifuge (page 29) or a vacuum manifold (page 52).

All protocols achieve the same cytosine conversion rates and lead to equal DNA recoveries after purification of converted DNA, independent of DNA starting amounts.

QIAamp® and DNeasy® Kits enable purification of high-quality genomic DNA from a variety of sample types (including blood, tissue, body fluids, and FFPE tissues), that is highly suited for cytosine conversion using the EpiTect 96 Bisulfite Kit procedure. See pages 62–65 for ordering information.

Equipment and Reagents to Be 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.

- Ethanol (molecular biology grade, 96–100%)*
- Multichannel pipet and tips (we recommend pipet tips with aerosol barriers for preventing cross-contamination)
- Thermal cycler with heated lid (since the bisulfite reaction is not overlaid with mineral oil, only thermal cyclers with heated lids are suitable for this procedure)
- Optional: If the lid of the thermal cycler is not compatible with the provided EpiTect cover foils, cap strips can be used (available from ABgene® [8 Flat Cap Strips, cat. no. AB-0783 or 8 Domed Cap Strips, cat. no. AB-0265])†
- Optional: Heating block, thermomixer, or heated orbital incubator (see step 1 of each protocol)

Spin protocols

- A centrifuge with 96-well plate rotor (e.g., Centrifuge 4–16S or Centrifuge 4–16KS [see www.qiagen.com for catalog no.] with Plate Rotor 2 x 96 [cat. no. 81031])

Vacuum protocols

- QIAvac 96 vacuum manifold (cat. no. 19504) or equivalent
- Vacuum Pump (cat. no. 84020) or equivalent pump capable of producing a vacuum of –800 to –900 mbar
- Elution microplate adapter for securely fixing the elution plate in the vacuum manifold during the elution step (e.g., Sarstedt PCR RackSystem [cat. no. 95.988])†
- Recommended: Vacuum Regulator (cat. no. 19530) for monitoring and easy release of vacuum pressure

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Yield and size of DNA

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

Using the standard protocol, the EpiTect 96 Bisulfite Kit is suited for DNA input amounts ranging from 1 ng to 2 µg, with high levels of DNA recovery throughout this range.

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.

If purifying bisulfite-treated DNA originating from very small sample amounts, such as biopsies and FFPE tissues, we strongly recommend adding carrier RNA to Buffer BL (see "Things to do before starting", pages 14, 19, 24, 29, 34, 40, 46, and 52).

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

Starting material

The 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, please linearize the DNA first due to the very quick reannealing of the single-stranded DNA after the denaturation step.

When working with fragmented DNA or already restriction digested DNA, we recommend using the protocol "Sodium Bisulfite Conversion of Unmethylated Cytosines in Small Amounts of Fragmented DNA using a Centrifuge", on page 29, which can also be performed on a vacuum manifold (page 52).

Handling of the EpiTect 96 Plate

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling EpiTect 96 Plates to avoid cross-contamination between sample preps:

- Carefully pipet the sample or solution into the EpiTect 96 Plate without wetting the rim of the wells. Avoid touching the EpiTect 96 Plate membrane with the pipet tip.
- Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips.
- Open sealed EpiTect 96 Plates carefully, and take care to avoid generating aerosols.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Centrifugation

Centrifugation of EpiTect 96 Plates is performed at 5800 x *g* (approximately 6000 rpm) unless otherwise stated. All centrifugation steps should be carried out at room temperature (15–25°C).

Note: If using a centrifuge with adjustable temperature (e.g. Centrifuge 4–16KS), set the temperature to 40°C for the drying step.

Adhesive tape

To prevent cross-contamination, AirPore Tape Sheets should be used to seal EpiTect 96 Plates prior to each centrifugation step, with the exception of the drying step (step 17 [pages 18 and 23] and step 18 [page 28 and 33]). In addition, Tape Pads should be used to cover the plate prior to storage of the samples. Used wells should be labeled using a waterproof marker pen while unused wells should be covered using an AirPore Tape Sheet cut to size. The EpiTect 96 Plate can then be stored in the blister pack in which it was supplied. Before starting the next run, remove the tape and cover the previously used wells with fresh tape.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA using a Centrifuge

This standard protocol should be used to process 1 ng – 2 µg DNA in a volume of up to 20 µl.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, dissolved Bisulfite Mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Add 120 ml ethanol (96–100%) to Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.
- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. Carrier RNA is not necessary if >100 ng DNA is used. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.
- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 1. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 20 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 1. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng–2 µg)	Variable* (maximum 20 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	35
Total volume	**140 µl**

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

3. **Seal the EpiTect Conversion Plate using EpiTect Cover Foil (provided) and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix, indicating sufficient mixing and correct pH for DNA bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User", page 11).

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

   The complete cycle should take approximately 5 h.

   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	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions at the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Place an EpiTect 96 Plate on top of an S-Block. Mark the plate for later identification.**

8. **Dispense 560 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 14) into the required wells of the EpiTect 96 Plate.**

   Note: Carrier RNA is not necessary when using >100 ng genomic DNA template.

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.

9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reaction will not affect the performance or yield of the reaction.

10. **Seal the EpiTect 96 Plate with an AirPore Tape Sheet (provided). Load the S-Block and EpiTect 96 Plate into the centrifuge plate holder, and then place the holder into the rotor bucket. Centrifuge at 5800 x *g* for 1 min.**

    Note: AirPore Tape Sheets help prevent cross contamination; however, their use is optional.

11. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**
12. **Remove the AirPore Tape Sheet. Carefully add 250 µl Buffer BD to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

13. **Centrifuge at 5800 x *g* for 1 min.**
14. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**
15. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min.**
16. **Remove the AirPore Tape Sheet. Carefully add 250 µl ethanol (96–100%) to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min.**

17. **Dispose of the S-Block appropriately. Remove the AirPore Tape Sheet and place the EpiTect 96 Plate on top of an EpiTect Elution Plate (provided). Centrifuge at 5800 x *g* for 15 min.**

    Note: If using a centrifuge with adjustable temperature, set the temperature to 40°C. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample.

18. **Place the EpiTect 96 Plate on top of a new EpiTect Elution Plate (provided).**

19. **To elute DNA, add 70 µl Buffer EB to each sample using a multichannel pipet.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of the EpiTect membrane.

20. **Centrifuge at 5800 x *g* for 1 min. Seal the elution plate for storage using the Tape Pads (provided).**

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of1 µl eluate should be sufficient for PCR amplification.

    Note: If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA from Low-Concentration Solutions using a Centrifuge

This protocol is optimized for low concentrations of DNA, which enables use of larger input volumes. With this protocol, 1–500 ng DNA in a maximum volume of 40 µl can be processed.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, dissolved Bisulfite Mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.
- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. Carrier RNA is not necessary if >100 ng DNA is used. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.

- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 3. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 40 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 3. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng–500 ng)	Variable* (maximum 40 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	15
Total volume	**140 µl**

* The combined volume of DNA solution and RNase-free water must total 40 µl.

3. **Seal the EpiTect Conversion Plate using EpiTect Cover Foil (provided) and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix, indicating sufficient mixing and correct pH for DNA bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User", page 11).

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

   The complete cycle should take approximately 5 h.

   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 4. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions at the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Place an EpiTect 96 Plate on top of an S-Block. Mark the plate for later identification.**
8. **Dispense 560 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 19) into the required wells of the EpiTect 96 Plate.**

   Note: Carrier RNA is not necessary when using >100 ng genomic DNA template.

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.
9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.
10. **Seal the EpiTect 96 Plate with an AirPore Tape Sheet (provided). Load the S-Block and EpiTect 96 Plate into the centrifuge plate holder, and then place the holder into the rotor bucket. Centrifuge at 5800 x *g* for 1 min.**

    Note: AirPore Tape Sheets help prevent cross contamination; however, their use is optional.
11. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**

12. **Remove the AirPore Tape Sheet. Carefully add 250 µl Buffer BD to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

13. **Centrifuge at 5800 x *g* for 1 min.**
14. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**
15. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min.**
16. **Remove the AirPore Tape Sheet. Carefully add 250 µl ethanol (96–100%) to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min.**
17. **Dispose of the S-Block appropriately. Remove the AirPore Tape Sheet and place the EpiTect 96 Plate on top of an EpiTect Elution Plate (provided). Centrifuge at 5800 x *g* for 15 min.**

    Note: If using a centrifuge with adjustable temperature, set the temperature to 40°C. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample.

18. **Place the EpiTect 96 Plate on top of a new EpiTect Elution Plate (provided).**
19. **To elute DNA, add 70 µl Buffer EB to each sample using a multichannel pipet.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

20. **Centrifuge at 5800 x *g* for 1 min. Seal the elution plate for storage using the Tape Pads (provided).**

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of 1 µl eluate should be sufficient for PCR amplification.

    Note: If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA Isolated from FFPE Tissue Samples using a Centrifuge

This protocol is optimized for processing DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. The samples should be extracted using an appropriate kit (e.g., QIAamp DNA Micro Kit, DNeasy 96 Tissue Kit, or EZ1® DNA Tissue Kit) prior to use in this procedure. An optimized binding step during the cleanup stage facilitates the binding of DNA from fixed tissues or otherwise degraded DNA. This protocol allows processing of 1 ng – 2 µg DNA in a volume of up to 20 µl.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, dissolved Bisulfite mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.
- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.

- Equilibrate samples and buffers to room temperature.
- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 5. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 20 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 5. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng – 2 µg)	Variable* (maximum 20 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	35
Total volume	**140 µl**

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

3. **Seal the EpiTect Conversion Plate using the EpiTect Cover Foil (provided) and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix, indicating sufficient mixing and correct pH for DNA bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User", page 11).

4. **Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 6.**

   The complete cycle should take approximately 5 h.

   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 6. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions in the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Place an EpiTect 96 Plate on top of an S-Block. Mark the plate for later identification.**
8. **Dispense 310 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 24) into the required wells of the EpiTect 96 Plate.**

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.
9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.
10. **Add 250 µl ethanol (molecular biology grade, 96–100%) to each sample and mix with the Buffer BL–bisulfite reaction solution by pipetting up and down 4 times.**
11. **Seal the EpiTect 96 Plate with an AirPore Tape Sheet (provided). Load the S-Block and EpiTect 96 Plate into the centrifuge plate holder, and then place the holder into the rotor bucket. Centrifuge at 5800 x *g* for 1 min.**

    Note: AirPore Tape Sheets help prevent cross contamination; however, their use is optional.
12. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**

13. **Remove the AirPore Tape Sheet. Carefully add 250 µl Buffer BD to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

14. **Centrifuge at 5800 x *g* for 1 min.**
15. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**
16. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape sheet. Centrifuge at 5800 x *g* for 1 min.**
17. **Remove the AirPore Tape Sheet. Carefully add 250 µl ethanol (96–100%) to each sample and seal the EpiTect 96 Plate with a new AirPore Tape sheet. Centrifuge at 5800 x *g* for 1 min.**
18. **Dispose of the S-Block appropriately. Remove the AirPore Tape Sheet and place the EpiTect 96 Plate on top of an EpiTect Elution Plate (provided). Centrifuge at 5800 x *g* for 15 min.**

    Note: If using a centrifuge with adjustable temperature, set the temperature to 40°C. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample.

19. **Place the EpiTect 96 Plate on top of a new elution plate (provided).**
20. **To elute DNA, add 70 µl Buffer EB to each sample using a multichannel pipet.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

21. **Centrifuge at 5800 x *g* for 1 min. Seal the elution plate for storage using the Tape Pads (provided).**

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of 1 µl eluate should be sufficient for PCR amplification.

    Note: If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in Small Amounts of Fragmented DNA using a Centrifuge

This protocol is designed for processing small amounts of DNA, which may also be fragmented. An optimized binding step in the cleanup stage facilitates binding of DNA. Less than 500 pg DNA in a volume of up to 40 µl can be processed using this protocol.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, dissolved Bisulfite mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.
- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.

- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 7. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 40 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 7. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (up to 500 pg)	Variable* (maximum 40 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	15
Total volume	**140 µl**

* The combined volume of DNA solution and RNase-free water must total 40 µl.

3. **Seal the EpiTect Conversion Plate using the EpiTect Cover Foil (provided) and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix, indicating sufficient mixing and correct pH for DNA bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User", page 11).

4. **Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 8.**

   The complete cycle should take approximately 5 h.

   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 8. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions in the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Place an EpiTect 96 Plate on top of an S-Block. Mark the plate for later identification.**
8. **Dispense 310 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 29) into the required wells of the EpiTect 96 Plate.**

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.
9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.
10. **Add 250 µl ethanol (molecular biology grade, 96–100%) to each sample and mix with the Buffer BL–bisulfite reaction solution by pipetting up and down 4 times.**
11. **Seal the EpiTect 96 Plate with an AirPore Tape Sheet (provided). Load the S-Block and EpiTect 96 Plate into the centrifuge plate holder, and then place the holder into the rotor bucket. Centrifuge at 5800 x *g* for 1 min.**

    Note: AirPore Tape Sheets help prevent cross contamination; however, their use is optional.
12. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**

13. **Remove the AirPore Tape Sheet. Carefully add 250 µl Buffer BD to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

14. **Centrifuge at 5800 x *g* for 1 min.**
15. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape Sheet. Centrifuge at 5800 x *g* for 1 min. Carefully empty the S-Block.**
16. **Remove the AirPore Tape Sheet. Carefully add 500 µl Buffer BW to each sample and seal the EpiTect 96 Plate with a new AirPore Tape sheet. Centrifuge at 5800 x *g* for 1 min.**
17. **Remove the AirPore Tape Sheet. Carefully add 250 µl ethanol (96–100%) to each sample and seal the EpiTect 96 Plate with a new AirPore Tape sheet. Centrifuge at 5800 x *g* for 1 min.**
18. **Dispose of the S-Block appropriately. Remove the AirPore Tape Sheet and place the EpiTect 96 Plate on top of an EpiTect Elution Plate (provided). Centrifuge at 5800 x *g* for 15 min.**

    Note: If using a centrifuge with adjustable temperature, set the temperature to 40°C. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample.

19. **Place the EpiTect 96 Plate on top of a new elution plate (provided).**
20. **To elute DNA, add 70 µl Buffer EB to each sample using a multichannel pipet.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

21. **Centrifuge at 5800 x *g* for 1 min. Seal the elution plate for storage using the Tape Pads (provided).**

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of1 µl eluate should be sufficient for PCR amplification.

    Note: If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA using a Vacuum Manifold

This standard protocol should be used to process 1 ng – 2 µg DNA in a volume of up to 20 µl.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, Bisulfite Mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- The QIAvac 96 can be operated using any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator). A vacuum pressure of between –800 to –900 mbar (–600 to –675 mm Hg) should be applied to the EpiTect 96 Plate.
- The vacuum should be switched off in between steps.

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.
- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.

- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. Carrier RNA is not necessary if >100 ng DNA is used. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.
- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.
- Seal unused wells of the EpiTect 96 Plate with Tape Pads, and place the EpiTect 96 Plate in the QIAvac Top Plate, making sure that the plate is seated securely.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 9, page 36. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 20 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 9. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng – 2 µg)	Variable* (maximum 20 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	35
Total volume	**140 µl**

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

3. **Seal the EpiTect Conversion Plate using the EpiTect Cover Foil and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User", page 11).

4. **Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 10, page 37.**

   The complete cycle should take approximately 5 h.

   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 10. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions in the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Prepare the vacuum manifold (e.g., QIAvac 96) and place an EpiTect 96 Plate securely onto it.**

   To prepare the QIAvac 96, place a waste tray inside the QIAvac base, and then place the QIAvac Top Plate squarely over the base. Attach the QIAvac 96 to a vacuum source.

   Seal unused wells of the EpiTect 96 Plate with Tape Pads.

8. **Dispense 560 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 34) into the required wells of the EpiTect 96 Plate.**

   Note: Carrier RNA is not necessary when using >100 ng genomic DNA template.

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.

9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.

10. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
11. **Carefully add 500 µl Buffer BW to each sample.**
12. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
13. **Carefully add 250 µl Buffer BD to each sample. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

14. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
15. **Carefully add 500 µl Buffer BW to each sample.**
16. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
17. **Repeat steps 15 and 16 once.**
18. **Carefully add 250 µl ethanol (96–100%) to each sample.**
19. **Switch on the vacuum source. After ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.**

    IMPORTANT: This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

20. **Switch off the vacuum source and ventilate the vacuum manifold slowly. Lift the QIAvac 96 Top Plate from the base (not the EpiTect 96 Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the EpiTect 96 Plate with clean absorbent paper.**

    This step removes residual ethanol from around the outlet nozzles and collars of the EpiTect 96 Plate. Residual ethanol may inhibit subsequent downstream reactions.

21. **Remove the waste tray and insert the vacuum manifold adapter for elution plates (see "Equipment and Reagents to Be Supplied by User", page 11). Place an elution plate directly onto the adapter and the top plate back onto the base.**

22. **To elute DNA, dispense 70 µl of Buffer EB and 10 µl Top Elute Fluid into each sample well of the EpiTect 96 Plate. Switch on the vacuum source a maximum of 1 min. Switch off the vacuum source and ventilate the vacuum manifold slowly.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of 1 µl eluate should be sufficient for PCR amplification.

    IMPORTANT: Small amounts of Top Elute Fluid can be eluted together with the DNA. Top Elute Fluid is clearly visible as a small bubble on top of the eluate. Top Elute Fluid is chemically inert and does not influence any downstream applications. However, Top Elute Fluid should not be transferred to the downstream reaction since it will reduce the amount of DNA intended to be transferred into the downstream reaction.

    Note: Seal the elution plate for storage using the Tape Pads (provided). If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA from Low-Concentration Solutions using a Vacuum Manifold

This protocol is optimized for low concentrations of DNA, which enables use of larger input volumes. With this protocol, 1–500 ng DNA in a maximum volume of 40 µl can be processed.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, Bisulfite Mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- The QIAvac 96 can be operated using any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator). A vacuum pressure of between –800 to –900 mbar (–600 to –675 mm Hg) should be applied to the EpiTect 96 Plate.
- The vacuum should be switched off in between steps.
- Seal unused wells of the EpiTect 96 Plate with Tape Pads, and place the EpiTect 96 Plate in the QIAvac Top Plate, making sure that the plate is seated securely.

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.

- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. Carrier RNA is not necessary if >100 ng DNA is used. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.
- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 11, page 42. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 40 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 11. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng – 500 ng)	Variable* (maximum 40 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	15
Total volume	**140 µl**

* The combined volume of DNA solution and RNase-free water must total 40 µl.

3. **Seal the EpiTect Conversion Plate using the EpiTect Cover Foil and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User" page 11).

4. **Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 12, page 43.**

   The complete cycle should take approximately 5 h.

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

Table 12. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions in the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Prepare the vacuum manifold (e.g., QIAvac 96) and place an EpiTect 96 Plate securely onto it.**

   To prepare the QIAvac 96, place a waste tray inside the QIAvac base, and then place the QIAvac Top Plate squarely over the base. Attach the QIAvac 96 to a vacuum source.

   Seal unused wells of the EpiTect 96 Plate with Tape Pads.

8. **Dispense 560 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 40) into the required wells of the EpiTect 96 Plate.**

   Note: Carrier RNA is not necessary when using >100 ng genomic DNA template.

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.

9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.

10. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
11. **Carefully add 500 µl Buffer BW to each sample.**
12. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
13. **Carefully add 250 µl Buffer BD to each sample. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

14. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
15. **Carefully add 500 µl Buffer BW to each sample.**
16. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
17. **Repeat steps 15 and 16 once.**
18. **Carefully add 250 µl ethanol (96–100%) to each sample.**
19. **Switch on the vacuum source. After ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.**

    IMPORTANT: This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

20. **Switch off the vacuum source and ventilate the vacuum manifold slowly. Lift the QIAvac 96 Top Plate from the base (not the EpiTect 96 Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the EpiTect 96 Plate with clean absorbent paper.**

    This step removes residual ethanol from around the outlet nozzles and collars of the EpiTect 96 Plate. Residual ethanol may inhibit subsequent downstream reactions.

21. **Remove the waste tray and insert the vacuum manifold adapter for elution plates (see "Equipment and Reagents to Be Supplied by User", page 11). Place an EpiTect Elution Plate directly onto the adapter and the top plate back onto the base.**

22. **To elute DNA, dispense 70 µl of Buffer EB and 10 µl Top Elute Fluid into each sample well of the EpiTect 96 Plate. Switch on the vacuum source for a maximum of 1 min. Switch off the vacuum source and ventilate the vacuum manifold slowly.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of 1 µl eluate should be sufficient for PCR amplification.

    IMPORTANT: Small amounts of Top Elute Fluid can be eluted together with the DNA. Top Elute Fluid is clearly visible as a small bubble on top of the eluate. Top Elute Fluid is chemically inert and does not influence any downstream applications. However, Top Elute Fluid should not be transferred to the downstream reaction since it will reduce the amount of DNA intended to be transferred into the downstream reaction.

    Note: Seal the elution plate for storage using the Tape Pads (provided). If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA from FFPE Tissue Samples using a Vacuum Manifold

This protocol is optimized for processing highly degraded DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. The samples should be purified using an appropriate kit (e.g., QIAamp DNA Micro Kit, DNeasy 96 Tissue Kit, or EZ1 DNA Tissue Kit) prior to use in this procedure. An optimized binding step in the cleanup stage facilitates binding DNA from fixed tissues or otherwise degraded DNA. With this protocol, 1 ng – 2 µg DNA in a volume of up to 20 µl can be processed.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, Bisulfite Mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- The QIAvac 96 can be operated using any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator). A vacuum pressure of between –800 to –900 mbar (–600 to –675 mm Hg) should be applied to the EpiTect 96 Plate.
- The vacuum should be switched off in between steps.
- Seal unused wells of the EpiTect 96 Plate with Tape Pads, and place the EpiTect 96 Plate in the QIAvac Top Plate, making sure that the plate is seated securely.

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.

- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.
- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 13, page 48. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 20 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 13. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng – 2 µg)	Variable* (maximum 20 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	35
Total volume	**140 µl**

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

3. **Seal the EpiTect Conversion Plate using the EpiTect Cover Foil and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: The DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User" page 11).

4. **Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 14, page 49.**

   The complete cycle should take approximately 5 h.

   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 14. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions in the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Prepare the vacuum manifold (e.g., QIAvac 96) and place an EpiTect 96 Plate securely onto it.**

   To prepare the QIAvac 96, place a waste tray inside the QIAvac base, and then place the QIAvac Top Plate squarely over the base. Attach the QIAvac 96 to a vacuum source.

   Seal unused wells of the EpiTect 96 Plate with Tape Pads.
8. **Dispense 310 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 46) into the required wells of the EpiTect 96 Plate.**

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.

9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.

10. **Add 250 µl ethanol (96–100%) to each sample and mix with the Buffer BL–bisulfite reaction solution by pipetting up and down 4 times.**
11. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
12. **Carefully add 500 µl Buffer BW to each sample.**
13. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
14. **Carefully add 250 µl Buffer BD to each sample. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

15. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
16. **Carefully add 500 µl Buffer BW to each sample.**
17. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
18. **Repeat steps 16 and 17.**
19. **Carefully add 250 µl ethanol (96–100%) to each sample.**
20. **Switch on the vacuum source. After ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.**

    Important: This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

21. **Switch off the vacuum source and ventilate the vacuum manifold slowly. Lift the QIAvac 96 Top Plate from the base (not the EpiTect 96 Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the EpiTect 96 Plate with clean absorbent paper.**

    This step removes residual ethanol from around the outlet nozzles and collars of the EpiTect 96 Plate. Residual ethanol may inhibit subsequent downstream reactions.

22. **Remove the waste tray and insert the vacuum manifold adapter for elution plates (see "Equipment and Reagents to Be Supplied by User", page 11). Place an EpiTect Elution Plate directly onto the adapter and the top plate back on the base.**
23. **To elute DNA, dispense 70 µl of Buffer EB and 10 µl Top Elute Fluid into each sample well of the EpiTect 96 Plate, and switch on the vacuum source for a maximum of 1 min. Switch off the vacuum source and ventilate the vacuum manifold slowly.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of 1 µl eluate should be sufficient for PCR amplification.

    IMPORTANT: Small amounts of Top Elute Fluid can be eluted together with the DNA. Top Elute Fluid is clearly visible as a small bubble on top of the eluate. Top Elute Fluid is chemically inert and does not influence any downstream applications. However, Top Elute Fluid should not be transferred to the downstream reaction since it will reduce the amount of DNA intended to be transferred into the downstream reaction.

    Note: Seal the elution plate for storage using the Tape Pads (provided). If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

Protocol: Sodium Bisulfite Conversion of Unmethylated Cytosines in Small Amounts of Fragmented DNA using a Vacuum Manifold

This protocol is designed for processing small amounts of DNA, which may also be fragmented. An optimized binding step in the cleanup stage facilitates binding of DNA. Less than 500 pg DNA in a volume of up to 40 µl can be processed using this protocol.

Important points before starting

- Each aliquot of Bisulfite Mix is sufficient for 96 conversion reactions. If converting fewer than 96 DNA samples, Bisulfite Mix can be stored at –20°C for up to 4 weeks without any loss in performance.
- DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.
- The QIAvac 96 can be operated using any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator). A vacuum pressure of between –800 to –900 mbar (–600 to –675 mm Hg) should be applied to the EpiTect 96 Plate.
- The vacuum should be switched off in between steps.
- Seal unused wells of the EpiTect 96 Plate with Tape Pads, and place the EpiTect 96 Plate in the QIAvac Top Plate, making sure that the plate is seated securely.

Things to do before starting

- Add 120 ml ethanol (96–100%) to the bottle containing Buffer BW and store at room temperature (15–25°C). Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C. Invert the bottle several times before starting the procedure and make sure to close the bottle immediately after use. White precipitates may form in the Buffer BD–ethanol mix after some storage time. These precipitates will not affect the performance of Buffer BD. However, avoid transferring precipitates to the EpiTect 96 Plate.

- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly by vortexing. Split the dissolved carrier RNA into conveniently sized aliquots (e.g., 675 µl aliquots for processing 96 reactions) and store at –20°C. Aliquots can be stored for up to 1 year. Carrier RNA enhances binding of DNA to the EpiTect 96 Plate, especially if there are very few target molecules in the sample.
- Add 310 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. If Buffer BL contains precipitates, dissolve by heating (maximum 70°C) with gentle agitation.
- Equilibrate samples and buffers to room temperature.
- Optional: set a thermomixer, heating block, or heated orbital incubator to 60°C for use in step 1.

Procedure

Bisulfite DNA conversion

1. **Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.**

   Note: If necessary, heat the Bisulfite Mix–RNase-free water solution to 60°C and vortex again.

   Note: Do not place dissolved Bisulfite Mix on ice.

2. **Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 15, page 54. Add each component in the order listed.**

   Note: The combined volume of DNA solution and RNase-free water must total 40 µl.

   Note: If using a multichannel pipet to dispense DNA Protect Buffer, use the provided EpiTect DNA Protect Buffer Reservoir. Commonly used polystyrene reservoirs are sensitive to the solvent in this buffer.

Table 15. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (up to 500 pg)	Variable* (maximum 40 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	15
Total volume	**140 µl**

* The combined volume of DNA solution and RNase-free water must total 40 µl.

3. **Seal the EpiTect Conversion Plate using the EpiTect Cover Foil and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).**

   Note: The DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix (step 2), indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

   Note: If the lid of the thermal cycler is not compatible with the provided EpiTect Cover Foils, cap strips can be used (see "Equipment and Reagents to Be Supplied by User" page 11).

4. **Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 16, page 55.**

   The complete cycle should take approximately 5 h.

   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 16. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min (2 h 55 min)	60°C
Hold	Indefinite*	20°C

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

5. **Place the EpiTect Conversion Plate 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.

   Converted DNA can be left in the thermal cycler overnight at 20°C without any loss of performance.

   At this point, samples can be stored at –20°C for up to 24 h. Before further processing, the samples must be heated to 60°C and mixed by vortexing to dissolve precipitates. After vortexing, centrifuge the plate briefly at 650 x *g* to collect all solutions in the bottom of the wells.

Cleanup of bisulfite converted DNA

6. **Briefly centrifuge the EpiTect Conversion Plate containing the bisulfite reactions at 650 x *g*.**
7. **Prepare the vacuum manifold (e.g., QIAvac 96) and place an EpiTect 96 Plate securely onto it.**

   To prepare the QIAvac 96, place a waste tray inside the QIAvac base, and then place the QIAvac Top Plate squarely over the base. Attach the QIAvac 96 to a vacuum source.

   Seal unused wells of the EpiTect 96 Plate with Tape Pads.
8. **Dispense 310 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA (see "Things to do before starting", page 52) into the required wells of the EpiTect 96 Plate.**

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.

9. **Transfer the complete bisulfite reactions from step 6 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.**

   Precipitates in the bisulfite reactions will not affect the performance or yield of the reaction.

10. **Add 250 µl ethanol (96–100%) to each sample and mix with the Buffer BL–bisulfite reaction solution by pipetting up and down 4 times.**
11. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
12. **Carefully add 500 µl Buffer BW to each sample.**
13. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
14. **Carefully add 250 µl Buffer BD to each sample. Incubate for 15 min at room temperature.**

    If there are precipitates in Buffer BD, avoid transferring them to the plate.

    IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.

    Note: It is important to close the lids of the spin columns before incubation.

15. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
16. **Carefully add 500 µl Buffer BW to each sample.**
17. **Switch on the vacuum source. After all liquid has passed through the membrane, switch off the vacuum source.**
18. **Repeat steps 16 and 17.**
19. **Carefully add 250 µl ethanol (96–100%) to each sample.**
20. **Switch on the vacuum source. After ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.**

    Important: This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

21. **Switch off the vacuum source and ventilate the vacuum manifold slowly. Lift the QIAvac 96 Top Plate from the base (not the EpiTect 96 Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the EpiTect 96 Plate with clean absorbent paper.**

    This step removes residual ethanol from around the outlet nozzles and collars of the EpiTect 96 Plate. Residual ethanol may inhibit subsequent downstream reactions.

22. **Remove the waste tray and insert the vacuum manifold adapter for elution plates (see "Equipment and Reagents to Be Supplied by User", page 11). Place an EpiTect Elution Plate directly onto the adapter and the top plate back on the base.**

23. **To elute DNA, dispense 70 µl of Buffer EB and 10 µl Top Elute Fluid into each sample well of the EpiTect 96 Plate, and switch on the vacuum source for a maximum of 1 min. Switch off the vacuum source and ventilate the vacuum manifold slowly.**

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of each EpiTect membrane.

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer. A volume of 1 µl eluate should be sufficient for PCR amplification.

    IMPORTANT: Small amounts of Top Elute Fluid can be eluted together with the DNA. Top Elute Fluid is clearly visible as a small bubble on top of the eluate. Top Elute Fluid is chemically inert and does not influence any downstream applications. However, Top Elute Fluid should not be transferred to the downstream reaction since it will reduce the amount of DNA intended to be transferred into the downstream reaction.

    Note: Seal the elution plate for storage using the Tape Pads (provided). If the purified DNA is to be stored for up to 24 h, we recommend storage at 2–8°C. For storage longer than 24 h, we recommend storage at –20°C. At –20°C, DNA converted and purified using the EpiTect 96 Bisulfite Kit can be stored for at least 3 years* without decrease of quality or conversion.

* Investigations into longer storage of converted DNA are ongoing. Contact QIAGEN for more information.

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 protocol in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Little or no DNA recovery in purification step	
a) Carrier RNA not added to Buffer BL	Prepare carrier RNA and add to Buffer BL, as described in "Things to do before starting", pages 14, 19, 24, 29, 34, 40, 46, and 52.
b) Buffer BW or Buffer BD prepared incorrectly	Check that Buffer BW and BD concentrates were diluted with the correct volumes of ethanol (96–100%). Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
c) Buffer BW or BD prepared with 70% ethanol	Check that Buffer BW and BD concentrates were diluted with 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
d) Buffer BW and Buffer BD used in the wrong order	Ensure that Buffer BW and Buffer BD are used in the correct order in the protocol.
e) Sample not completely passed through the membrane	Centrifuge at 5800 x *g* for 1 min or until the entire sample has passed through the membrane. If using a vacuum manifold, apply vacuum until all liquid has passed through the membrane.
f) Buffer BL contains precipitates	Check Buffer BL for precipitate. Dissolve by heating (maximum 70°C) with gentle agitation.
Low conversion rate	
a) Bisulfite reaction components not added in the correct order	Ensure that DNA, Bisulfite Mix, and DNA Protect Buffer are added in the order indicated in Tables 1, 3, 5, 7, 9, 11, 13, and 15.

	Comments and suggestions
b) Incorrect thermal cycling conditions used	Use the thermal cycling conditions given in Tables 2, 4, 6, 8, 10, 12, 14, and 16.
c) 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. Ensure that sample DNA is purified using an appropriate kit (see ordering information, pages 62–65 for suitable QIAGEN kits for DNA purification).
d) 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 DNA for standard applications (pages 14 and 34), 1 ng to 500 ng for DNA in low-concentration solutions (pages 19 and 40), or 1 ng to 2 µg for DNA isolated from FFPE tissues (pages 24 and 46) or fragmented DNA (pages 29 and 52).
e) Bisulfite Mix stored incorrectly	Dissolved Bisulfite Mix can be stored at –20°C for 4 weeks.
f) DNA Protect Buffer not added	Upon addition of DNA Protect Buffer, the DNA–Bisulfite Mix solution should turn from green to blue indicating sufficient mixing and the correct pH for DNA binding to the EpiTect 96 Plate. If this color change does not occur, repeat the reaction ensuring that DNA Protect Buffer has been added.
Poor results in downstream methylation-specific PCR	
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.

	Comments and suggestions
b) Failure of conversion reaction	The starting DNA was not sufficiently pure. Ensure that only high-quality DNA is used for the conversion reaction. See ordering information, pages 62–65 for suitable QIAGEN kits for DNA purification. 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.
c) Eluate contains residual ethanol (from Buffer BW or ethanol washing step)	Ensure the duration of the drying step is sufficient and that the plate is not sealed using an AirPore Tape Sheet. If using a centrifuge with adjustable temperature settings, set the temperature to 40°C. If a temperature controlled centrifuge is not available, we recommend placing the EpiTect 96 Plate in an incubator at 65°C for 15 min to evaporate residual ethanol. If the DNA concentration is sufficiently high, decrease the volume of eluate transferred to the PCR.
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 one year, and a change in color within this time has no influence on performance.
b) Precipitates in Buffer BD	There may be slight clouding and/or insoluble precipitates in Buffer BD during storage. Buffer BD is stable at 2–8°C for one year, and a precipitate within this time has no influence on performance. Precipitates should not be transferred onto the membrane.

	Comments and suggestions
General handling	
Vacuum pressure too high or low	Using a vacuum pressure that is too high may damage the membrane. Using a vacuum pressure that is too low may cause reduced DNA yield and purity. Use a vacuum regulator (see ordering information on page 62) to adjust the pressure to –800 to –900 mbar for all vacuum steps.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
EpiTect Bisulfite 96 Kit (2)	2 x EpiTect Bisulfite 96 Plates, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59110
EpiTect Bisulfite Kit (48)	48 EpiTect Bisulfite Spin Columns, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59104
Related products		
EpiTect Whole Bisulfitome Kit — for amplification of bisulfite converted DNA		
EpiTect Whole Bisulfitome Kit (25)*	REPLI-g® Midi DNA Polymerase, EpiTect WBA Reaction Buffer, Nuclease free water	59203
EpiTect MSP Kit — for highly accurate methylation-specific PCR without optimization		
EpiTect MSP Kit (25)*	EpiTect MSP Master Mix for 25x50 µl reactions	59303
EpiTect MethyLight PCR Kits — for real time quantification of methylation status		
EpiTect MethyLight PCR Kit (200)*	Master Mix for Methylation-specific Real-Time PCR analysis, 200 x50 µl reactions	59436
EpiTect MethyLight PCR + ROX™ Vial Kit (200)*	Master Mix without ROX for Methylation-specific Real-Time PCR analysis, 200 x50 µl reactions	59496
EpiTect Control DNA — for evaluation of PCR primers used for methylation analysis		
EpiTect Control DNA, methylated (100)	Methylated and bisulfite converted human control DNA for 100 control PCR reactions	59655
EpiTect Control DNA, unmethylated (100)	Unmethylated and bisulfite converted human control DNA for 100 control PCR reactions	59665

* Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
EpiTect Control DNA (1000)	Unmethyletd human Control DNA for 1000 control PCR reactions	59568
EpiTect PCR Control DNA Set (100)	Human control DNA Set for 100 control PCR reactions	59695
EZ1 DNA Tissue Kit — for automated purification of high-quality DNA from 1–14 tissue samples using the EZ1 instruments		
EZ1 DNA Tissue Card	Preprogrammed card for purification of DNA using the BioRobot® EZ1	9015588
EZ1 Advanced DNA Tissue Card	Preprogrammed card for purification of DNA using the EZ1 Advanced	9018295
EZ1 Advanced XL DNA Tissue Card	Preprogrammed card for purification of DNA using the EZ1 Advanced XL	9018701
EZ1 DNA Paraffin Section Card	Preprogrammed card for BioRobot EZ1 paraffin section protocols	9015862
EZ1 Advanced DNA Paraffin Section Card	Preprogrammed card for purification of DNA using the EZ1 Advanced	9018298
EZ1 Advanced XL DNA Paraffin Section Card	Preprogrammed card for purification of DNA using the EZ1 Advanced XL	9018700
EZ1 DNA Tissue Kit (48)	For 48 DNA preps: 48 Reagent Cartridges (Tissue), Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K	953034
DNeasy Tissue Kits — for purification of total cellular DNA from animal tissues and cells, yeast, or bacteria		
DNeasy Tissue Kit (50)*	For 50 DNA minipreps: 50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
DNeasy 96 Tissue Kit (4)*	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 ml), Elution Microtubes RS, Caps, 96-well Plate Registers	69581

* Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
QIAamp DNA Micro Kit — for purification of genomic and mitochondrial DNA from small amounts of fresh or frozen blood, tissue, FFPE tissue, and dried blood spots		
QIAamp DNA Micro Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304
QIAamp DNA Mini Kit — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA from a wide variety of samples		
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Blood Kit — for purification of genomic, mitochondrial, or viral DNA from blood and related body fluids		
QIAamp DNA Blood Mini Kit (50)*†	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
FlexiGene® DNA Kits — for purification of genomic DNA from whole blood, buffy coat, or cultured cells in a single tube		
FlexiGene DNA Kit (50)*	For purification of DNA from 50 ml whole blood: Buffers, QIAGEN Protease	51204
FlexiGene DNA AGF3000 Kit (640)	For automated purification of DNA from 640 whole blood samples using the AutoGenFlex STAR workstation: Buffers, QIAGEN Protease	51297
Gentra® Puregene® Blood Kit — for purification of archive-quality DNA from blood		
Gentra Puregene Blood Kit (3 ml)*	For 3 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158422

* Other kit sizes are available; see www.qiagen.com.

† Requires use of the QIAGEN 96-Well-Plate Centrifugation System.

Product	Contents	Cat. no.
EZ1 DNA Blood Kits — for automated purification of DNA from 1–14 blood samples using the EZ1 instruments		
EZ1 DNA Blood 200 µl Kit (48)	For 48 DNA preps: 48 Reagent Cartridges (Blood 200 µl), Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes (2 ml), Elution Tubes (1.5 ml)	951034
EZ1 DNA Blood 350 µl Kit (48)	For 48 DNA preps: 48 Reagent Cartridges (Blood 350 µl), Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes (2 ml), Elution Tubes (1.5 ml)	951054
EZ1 DNA Blood Card	Preprogrammed card for BioRobot EZ1 DNA Blood 200 µl and 350 µl Protocols	9015585
EZ1 Advanced DNA Blood Card	Preprogrammed card for purification of DNA using the EZ1 Advanced	9018293
EZ1 Advanced XL DNA Blood Card	Preprogrammed card for purification of DNA using the EZ1 Advanced XL	9018695
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate Base, Waste Tray, Plate Holder, Rack of collection microtubes (1.2 ml)	19504
Vacuum Regulator	For use with QIAvac manifolds	19530

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.

* Other kit sizes are available; see www.qiagen.com.

Notes

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