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# *therascreen*® MGMT Pyro® Kit Handbook

Σ 48

Version 1

IVD

For in vitro diagnostic use

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QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

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QIAGEN®

Sample & Assay Technologies

# QIAGEN Sample and Assay Technologies

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# Contents

# Intended Use

The *therascreen* MGMT Pyro Kit is an *in vitro* nucleic acid sequence-based detection test based on Pyrosequencing® technology for quantitative measurements of methylation status in exon 1 of the human MGMT gene in genomic DNA derived from human tissue samples.

The *therascreen* MGMT Pyro Kit is intended to be used as an adjunct to other prognostic factors and to provide clinicians with information to aid the selection of cancer patients more likely to benefit from chemotherapies. For in vitro diagnostic use.

For use only on the PyroMark® Q24 system. PyroMark Q24 systems include the following:

- The PyroMark Q24 instrument and the PyroMark Q24 MDx instrument.
- The PyroMark Q24 Vacuum Workstation and the PyroMark Q24 MDx Vacuum Workstation.
- The PyroMark Q24 Software (version 2.0) and PyroMark Q24 MDx Software (version 2.0).

The product is intended to be used by professional users, such as technicians and physicians who are trained in in vitro diagnostics procedures, molecular biological techniques, and the PyroMark Q24 system.

# Summary and Explanation

The *therascreen* MGMT Pyro Kit is intended for quantitative measurements of methylation in four CpG sites in exon 1 of the human MGMT gene (genomic sequence on chromosome 10 from 131,265,519 to 131,265,537: CGACGCCCGCAGGTCCTCG). Bisulfite converted genomic DNA is amplified by PCR and sequenced through the defined region in the forward direction (Figure 1). Sequences surrounding the defined positions serve as normalization and reference peaks for quantification and quality assessment of the analysis.

The product consists of PCR primer mix and sequencing primer, two vials of each. The primers are delivered in solution. Each vial contains 24 µl of primer or primer mix. The kit contains primers and reagents for amplification of the genes, plus buffers, primers, and reagents for quantitative methylation detection in real time using Pyrosequencing technology on the PyroMark Q24 System.

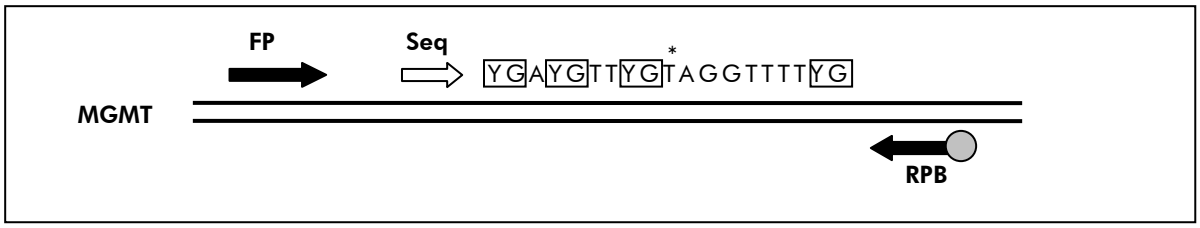


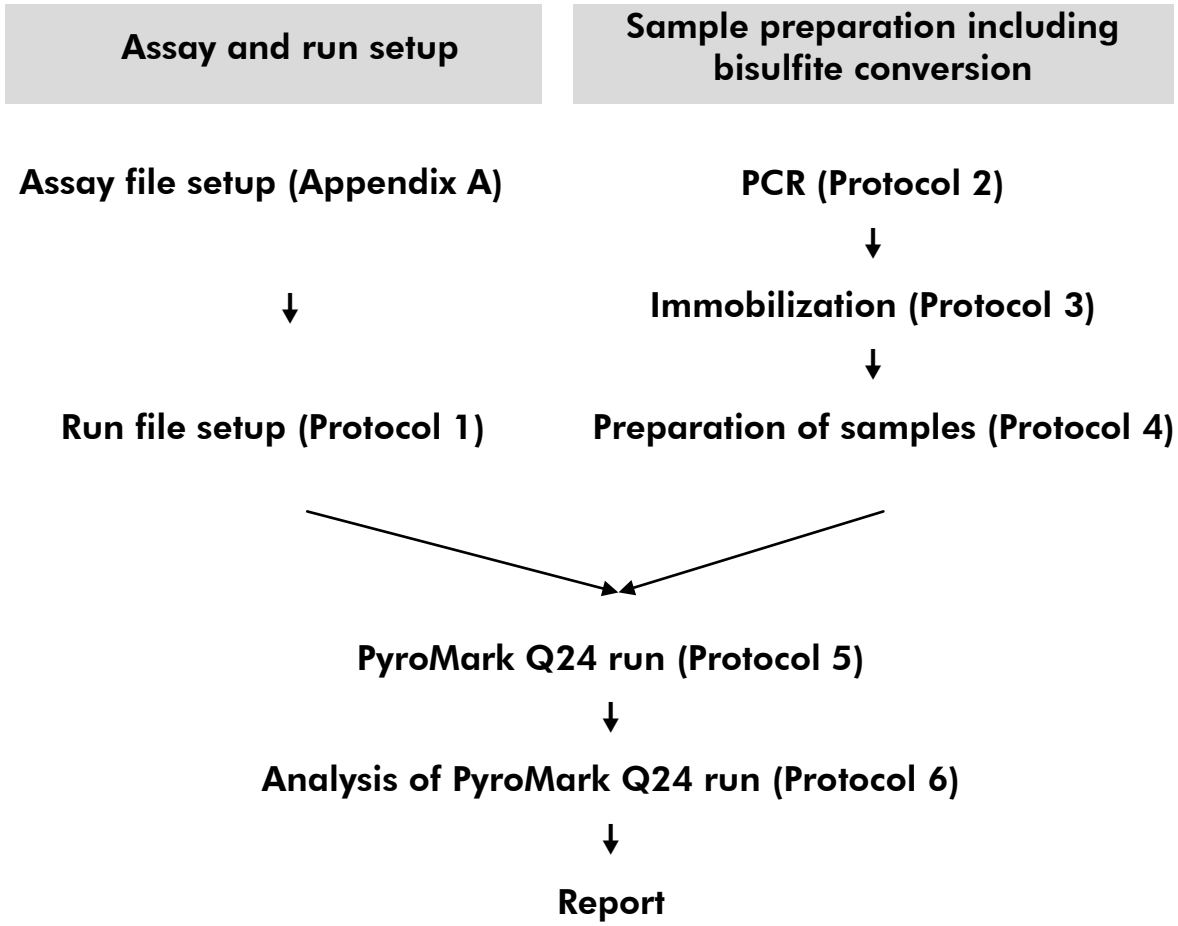

**Figure 1. Illustration of the MGMT assay.** The sequence indicated is the analyzed sequence after bisulfite conversion. Y indicates the potentially methylated sites and boxes indicate the analyzed CpG sites. The asterisk indicates the site for bisulfite conversion control. **FP**: Forward PCR primers; **RPB**: Reverse PCR primers (**B** indicates biotinylation); **Seq**: Sequencing primers.

# Principle of the Procedure

The workflow below illustrates the assay procedure. After PCR using primers targeting the defined region of exon 1, the amplicons are immobilized on Streptavidin Sepharose® High Performance beads. Single-stranded DNA is prepared and the sequencing primer annealed to the DNA. The samples are then analyzed on the PyroMark Q24 System using an assay setup file and a run file.

**Note:** The workflow has been slightly modified compared to the *PyroMark Q24 User Manual* (see "Protocol 4: Preparation of samples prior to Pyrosequencing analysis on the PyroMark Q24", page 21).

**Workflow of the *therascreen* MGMT Pyro procedure**




## Controls

Methylated Control DNA is included in the kit as a positive control for PCR and sequencing reactions. This control DNA is highly methylated and bisulfite converted. It is also recommended that a DNA sample derived from a healthy blood donor is included in every Pyrosequencing run for comparison. In addition, a negative control (without template DNA) should be included in every PCR setup.

# Materials Provided

## Kit contents

### *therascreen* MGMT Pyro Kit (box 1/2)

| ***therascreen* MGMT Pyro Kit** | **(48)** |
|---|---|
| **Catalog no.** | **971061** |
| **Number of reactions** | **48** |
| PCR Primer Mix MGMT | 2 x 24 µl |
| Seq Primer MGMT | 2 x 24 µl |
| PyroMark PCR Master Mix, 2x | 850 µl |
| CoralLoad® Concentrate, 10x | 1.2 ml |
| $H_2O$ | 3 x 1.9 ml |
| Methylated Control DNA, 10 ng/µl | 100 µl |

### *therascreen* Pyro buffers and reagents (box 2/2)

| ***therascreen* Pyro buffers and reagents** | |
|---|---|
| PyroMark Binding Buffer | 10 ml |
| PyroMark Annealing Buffer | 10 ml |
| PyroMark Denaturation Solution* | 250 ml |
| PyroMark Wash Buffer, 10x | 25 ml |
| Enzyme Mixture | 1 vial |
| Substrate Mixture | 1 vial |
| dATPαS | 1180 µl |
| dCTP | 1180 µl |
| dGTP | 1180 µl |
| dTTP | 1180 µl |
| Handbook HB | 1 |

* Contains sodium hydroxide.

## Materials Required but Not Provided

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.

- DNA isolation kit (see "DNA isolation and bisulfite conversion", page 13)
- Reagents for bisulfite conversion of DNA (see "DNA isolation and bisulfite conversion", page 13)
- Pipets (adjustable)*
- Sterile pipet tips (with filters for PCR setup)
- Benchtop microcentrifuge*
- Thermal cycler and appropriate PCR tubes
- Streptavidin Sepharose High Performance (GE Healthcare, cat. no. 17-5113-01; www.gelifesciences.com)
- PyroMark Q24 (cat. no. 9001513 or 9001514)*†
- PyroMark Q24 Software (cat. no. 9019062 or 9019063)†
- PyroMark Q24 Plate (cat. no. 979201)†
- PyroMark Q24 Cartridge (cat. no. 979202)†
- PyroMark Q24 Vacuum Workstation (cat. no. 9001515 or 9001517)*†
- Plate mixer* for immobilization to beads (see "Recommended plate mixers", page 10)
- Heating block* capable of attaining 80°C
- 24-well PCR plate or strips
- Strip caps
- High-purity water (Milli-Q® 18.2 MΩ x cm or equivalent)

  **Note**: Sufficient water is provided in the product for PCR, DNA immobilization, and for dissolving the Enzyme Mixture and the Substrate Mixture; additional high-purity water is required to dilute PyroMark Wash Buffer, 10x
- Ethanol (70%)‡

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

† CE-IVD-marked in accordance with EU Directive 98/79/EC. All other products listed are not CE-IVD-marked based on EU Directive 98/79/EC.

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

## Recommended plate mixers

The plate mixers shown in Table 1 are recommended for use with the *therascreen* MGMT Pyro Kit.

**Table 1. Plate mixers recommended for use with the *therascreen* MGMT Pyro Kit**

| Manufacturer | Product | Catalog number |
|---|---|---|
| Eppendorf | Thermomixer comfort (Basic device) | 5355 000.011 |
| | Thermoblock for MTP | 5363 000.012 |
| | Adapter plate for 96 x 0.2ml PCR tubes to insert in blocks for microtiter plates | 5363 007.009 |
| H+P Labortechnik GmbH | Variomag® Teleshake | 51410 (115 V=51410 U) |
| | Variomag Monoshake | 51110 (115 V=51110 U) |

# Warnings and Precautions

For In Vitro Diagnostic Use

## Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

The following hazard and precautionary statements apply to components of the *therascreen* MGMT Pyro Kit.

### PyroMark Denaturation Solution

Warning! Causes skin irritation. Causes serious eye irritation. May be corrosive to metals. Absorb spillage to prevent material damage. Keep only in original container. Wear protective gloves/ protective clothing/eye protection/face protection.

### PyroMark Enzyme Mixture

Contains: (R*,R*)-1,4-Dimercaptobutane-2,3-diol; acetic acid. Danger! Causes skin irritation. Causes serious eye damage. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Call a POISON CENTER or doctor/ physician. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/ face protection.

### PyroMark Substrate Mixture

Contains: acetic acid. Warning! Causes skin irritation. Causes serious eye irritation. If eye irritation persists: Get medical advice/ attention. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/ face protection.

## General precautions

The user should always pay attention to the following.

- Strict compliance with the user manual is required for optimal results. Dilution of the reagents, other than as described in this handbook, is not recommended and will result in a loss of performance.
- Note that the workflow has been slightly modified compared to the *PyroMark Q24 User Manual* (see "Protocol 4: Preparation of samples prior to Pyrosequencing analysis on the PyroMark Q24", page 21).
- The components of this product are sufficient to perform the 48 reactions in up to 5 independent runs.
- Use sterile pipet tips with filters (for PCR setup).

- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.
- Failed results are not a basis for judgment of methylational status.

## Reagent Storage and Handling

The *therascreen* MGMT Pyro Kit is shipped in two boxes. The *therascreen* MGMT Pyro Kit (box 1/2) is shipped on dry ice. PyroMark PCR Master Mix, CoralLoad Concentrate, Methylated Control DNA, and all primers should be stored at –30°C to –15°C upon arrival.

The *therascreen* Pyro buffers and reagents (box 2/2) containing buffers, Enzyme Mixture, Substrate Mixture, dATPαS, dCTP, dGTP, and dTTP (the reagents for Pyrosequencing analysis) are shipped on cool packs. These components should be stored at 2–8°C upon arrival. To minimize loss of activity, it is advisable to keep both the enzyme mixture and the substrate mixture in the vials supplied.

Reconstituted enzyme and substrate mixtures are stable for at least 10 days at 2–8°C. Reconstituted enzyme and substrate mixtures can be frozen and stored in their vials at –30°C to –15°C. Frozen reagents should not be subjected to more than 6 freeze–thaw cycles.

**Note**: Nucleotides should not be frozen.

The *therascreen* MGMT Pyro Kit is stable until the kit expiration date when stored under these conditions.

## Specimen Handling and Storage

All samples must be treated as potentially infectious material.

Specimen material is bisulfite converted human DNA extracted from blood or formalin-fixed paraffin-embedded (FFPE) samples.

Samples from humans undergoing heparin treatment must not be used. Blood samples that have been collected in tubes containing heparin as an anticoagulant should not be used. Heparin affects the PCR.

# Procedure

## DNA isolation and bisulfite conversion

The system performance has been established using the EZ1® DNA Tissue Kit and the QIAamp® DNA FFPE Tissue Kit for extraction of human DNA from formalin-fixed paraffin-embedded tumor samples. For the QIAamp DSP DNA Blood Mini Kit system, performance has been established using healthy donor blood samples partially spiked with tumor cells.

The kits from QIAGEN shown in Table 2 are recommended for DNA purification from the indicated human sample types for use with the *therascreen* MGMT Pyro Kit. Carry out the DNA purification according to the instructions in the kit handbooks.

For bisulfite conversion, the EpiTect® Bisulfite Kit (cat. no. 59104), EpiTect Plus FFPE Bisulfite Kit (cat. no. 59144), or EpiTect Plus DNA Bisulfite Kit (cat. no. 59124) from QIAGEN is recommended.

**Table 2. DNA purification kits recommended for use with the *therascreen* MGMT Pyro Kit**

| Sample material | Nucleic acid isolation kit | Catalog number (QIAGEN) |
|---|---|---|
| Paraffin-embedded tissue | QIAamp DNA FFPE Tissue Kit (50) | 56404 |
| | EZ1 DNA Tissue Kit (48)* | 953034 |
| Blood | QIAamp DSP DNA Blood Mini Kit† | 61104 |

* Follow the protocol for use with paraffin-embedded tissue. The EZ1 DNA Tissue Kit should be used in combination with the EZ1 Advanced (cat. no. 9001410 or 9001411) and the EZ1 Advanced DNA Paraffin Section Card (cat. no. 9018298), with the EZ1 Advanced XL (cat. no. 9001492) and the EZ1 Advanced XL DNA Paraffin Section Card (cat. no. 9018700), or with the BioRobot® EZ1 (cat. no. 9000705; no longer available) and the EZ1 DNA Paraffin Section Card (cat. no. 9015862).

† CE-IVD marked in accordance with EU Directive 98/79/EC.

## Protocol 1: Run setup for the PyroMark Q24 system

### Important point before starting

- If required, the LOB can be confirmed by using a healthy blood donor samples to generate a full plate of results. For details, consult CLSI Guideline EP17-A "Protocol for determination of limits of detection and limits of quantitation; approved guideline".

### Things to do before starting

- Create an Assay Setup as described in Appendix A, page 42. This needs to be done only once, before running the *therascreen* MGMT assay for the first time.

### Procedure

1. **Click  in the toolbar.**

   A new run file is created.

2. **Enter the run parameters (see "Run parameters", page 15).**
3. **Set up the plate by adding the assay to wells corresponding to the samples to analyze.**

   **Note**: A negative control sample (without template DNA) should be included in every PCR setup.

   **Note**: It is also recommended to include a control sample with DNA from a healthy blood donor in each Pyrosequencing run for comparison. A sample with Methylated Control DNA can be included as a positive control for the PCR and sequencing reactions (see "Controls", page 7).

4. **When the run is set up and ready to run on the PyroMark Q24 system, print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select "Pre Run Information" from the "Tools" menu and when the report appears click  .**
5. **Close the run file and copy it to a USB stick (supplied with the system) using Windows® Explorer.**

   The printed Pre Run Information can be used as a template for the sample setup (see "Protocol 3: Immobilization of PCR products to Streptavidin Sepharose High Performance beads", page 19).

   To run the plate on PyroMark Q24, see "Protocol 5: Running the PyroMark Q24", page 25.

### Run parameters

| | |
|---|---|
| Run name: | The name of the run is given when the file is saved. Renaming the file also changes the name of the run. |
| Instrument method: | Select the instrument method according to the cartridge that will be used for the run. See the instructions supplied with the products. |
| Plate ID: | **Optional**: Enter ID of the PyroMark Q24 Plate. |
| Bar code: | **Optional**: Enter a bar code number for the plate or, if you have a bar code reader connected to your computer, place the mouse cursor in the "Barcode" text box (by clicking the box) and scan the bar code. |
| Reagent ID: | **Optional**: Enter the lot numbers for the *therascreen* MGMT Pyro Kit box 1 and box 2 to be used. The lot number can be found on the product label.<br><br>**Note**: We recommend entering the lot number so that any unexpected problems with the *therascreen* MGMT Pyro Kit can be traced. |
| Run note: | **Optional**: Enter a note about the contents or purpose of the run. |

### Add assay files

To add an assay to a well, you can either:

- Right-click the well and select "Load Assay" from the context menu.
- Select the assay in the shortcut browser and click-and-drag the assay to the well.

A well is color-coded according to the assay loaded to the well.

### Enter sample IDs and notes

To enter a sample ID or note, select the cell and enter the text.

To edit a sample ID or note, either select the cell (the current contents will be selected) or double-click the cell.

## Protocol 2: PCR using the reagents supplied with the *therascreen* MGMT Pyro Kit

This protocol is for PCR amplification of a region of bisulfite converted DNA using the *therascreen* MGMT Pyro Kit.

### Important points before starting

- The HotStarTaq® DNA polymerase in the PyroMark PCR Master Mix requires an activation step of **15 minutes at 95°C.**
- Set up all reaction mixtures in an area separate from that used for DNA purification, adding template DNA to the PCR, PCR product analysis, or preparation of samples prior to Pyrosequencing analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Bisulfite converted DNA must be used as template DNA. The EpiTect Bisulfite Kit (cat. no. 59104), EpiTect Plus FFPE Bisulfite Kit (cat. no. 59144), or EpiTect Plus DNA Bisulfite Kit (cat. no. 59124) from QIAGEN are recommended.

### Things to do before starting

- Before opening the tube with PCR primer, centrifuge briefly to collect contents at the bottom of the tubes.
- Adjust the concentration of the sample DNA to 2–10 ng/µl, if necessary.

### Procedure

1. **Thaw all necessary components.**
   Mix well before use.
2. **Prepare a reaction mix according to Table 3.**
   The reaction mix typically contains all of the components needed for PCR, except the sample.

   Prepare a volume of reaction mix greater than that required for the total number of PCR assays to be performed.

**Table 3. Preparation of reaction mix**

| Component | Volume/reaction (µl) |
|---|---|
| PyroMark PCR Master Mix, 2x | 12.5 |
| CoralLoad Concentrate, 10x | 2.5 |
| PCR Primer Mix MGMT | 1.0 |
| Water ($H_2O$, supplied) | 4.0 |
| **Total volume** | **20.0** |

3. **Mix the reaction mix thoroughly and dispense 20 µl into each PCR tube.**

   It is not necessary to keep PCR tubes on ice since HotStarTaq DNA polymerase is inactive at room temperature.

4. **Add 5 µl bisulfite converted template DNA (10–50 ng of genomic DNA as measured before bisulfite conversion) to the individual PCR tubes (Table 4) and mix thoroughly.**

   **Note**: A negative control sample (without template DNA) should be included in every PCR setup.

   **Note**: It is also recommended to include a control sample with DNA from a healthy blood donor in each Pyrosequencing run for comparison. A sample with Methylated Control DNA can be included as a positive control for the PCR and sequencing reactions (see "Controls", page 7).

**Table 4. Preparation of PCR**

| Component | Volume/reaction (µl) |
|---|---|
| Reaction mix | 20 |
| Sample DNA | 5 |
| **Total volume** | **25** |

5. **Program the thermal cycler according to the manufacturer's instructions using the conditions outlined in Table 5.**

**Table 5. Optimized cycling protocol**

| | | | Comments |
|---|---|---|---|
| **Initial activation step:** | 15 minutes | 95°C | HotStarTaq DNA polymerase is activated by this heating step. |
| **3-step cycling:** | | | |
| Denaturation | 20 seconds | 95°C | |
| Annealing | 30 seconds | 53°C | |
| Extension | 20 seconds | 72°C | |
| Number of cycles | 42 | | |
| **Final extension:** | 5 minutes | 72°C | |

6. **Place the PCR tubes in the thermal cycler and start the cycling program.**
7. **After amplification, proceed with "Protocol 3: Immobilization of PCR products to Streptavidin Sepharose High Performance beads", page 19.**

## Protocol 3: Immobilization of PCR products to Streptavidin Sepharose High Performance beads

This protocol is for immobilization of template DNA to Streptavidin Sepharose High Performance (GE Healthcare) prior to analysis on the PyroMark Q24 system.

### Important points before starting

- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

### Procedure

1. **Gently shake the bottle containing Streptavidin Sepharose High Performance until it is a homogeneous solution.**
2. **Prepare a master mix for DNA immobilization according to Table 6.** Prepare a volume 10% greater than that required for the total number of reactions to be performed.

**Table 6. Master mix for DNA immobilization**

| Component | Volume/sample (µl) |
|---|---|
| Streptavidin Sepharose High Performance | 2 |
| PyroMark Binding Buffer | 40 |
| Water ($H_2O$, supplied) | 28 |
| **Total volume** | **70** |

3. **Add 70 µl of the master mix to wells of a 24-well PCR plate (or strips) as predefined in the run setup (see "Protocol 1: Run setup for the PyroMark Q24 system", page 14).**
4. **Add 10 µl of biotinylated PCR product from Protocol 2 to each well containing master mix, as predefined in the run setup (see "Protocol 2: PCR using the reagents supplied with the *therascreen* MGMT Pyro Kit", page 16).**

   The total volume per well should be 80 µl after addition of the master mix and PCR product.
5. **Seal the PCR plate (or strips) using strip caps.**

   Ensure that no leakage is possible between the wells.

6. **Agitate the PCR plate at room temperature (15–25°C) for 5–10 minutes at 1400 rpm.**

   During this step, prepare the PyroMark Q24 Vacuum Workstation for sample preparation as described in the *PyroMark Q24 User Manual*.

7. **Proceed immediately with "Protocol 4: Preparation of samples prior to Pyrosequencing analysis on the PyroMark Q24 ", page 21.**

   **Note**: Sepharose beads sediment quickly. Capturing of the beads must take place immediately following agitation.

   If more than 1 minute has elapsed since the plate (or strips) was agitated, agitate again for 1 minute before capturing the beads.

## Protocol 4: Preparation of samples prior to Pyrosequencing analysis on the PyroMark Q24

This protocol is for preparation of single-stranded DNA and annealing of the sequencing primer to the template prior to Pyrosequencing analysis on the PyroMark Q24.

### Important points before starting

- Add the sequencing primer in the same pattern as predefined for the plate in the run setup (see "Protocol 1: Run setup for the PyroMark Q24 system", page 14).
- The workflow has been slightly modified compared to the *PyroMark Q24 User Manual* (step 18). Do not shorten the time for cooling down the samples after heating to 80°C.
- Perform the function test for filter probes as described in the *PyroMark Q24 User Manual* on a regular basis and exchange filter probes if indicated.

### Things to do before starting

- Before opening the tube with sequencing primer, centrifuge briefly to collect contents at the bottom of the tubes.
- Place one PyroMark Q24 Plate Holder on a preheated heating block at 80°C for use in step 17. Leave a second PyroMark Q24 Plate Holder at room temperature (15–25°C) for use in step 18.
- PyroMark Wash Buffer is supplied as a 10x concentrate. Before using for the first time, add high-purity water to 25 ml 10x PyroMark Wash Buffer to achieve a final volume of 250 ml and obtain a 1x working solution.

  The 1x PyroMark Wash Buffer working solution is stable at 2–8°C until the marked expiration date.

### Procedure

1. **Dilute a sufficient amount of the sequencing primer, Seq Primer MGMT, in PyroMark Annealing Buffer as shown in Table 7.**

   Prepare a volume of diluted sequencing primer greater than that required for the total number of samples to be sequenced (for the number of samples + one extra).

**Table 7. Example dilution of the sequencing primer**

| Component | Volume/sample (µl) | Volume for 9 + 1 reactions (µl) |
|---|---|---|
| Seq Primer MGMT | 0.8 | 8.0 |
| PyroMark Annealing Buffer | 24.2 | 242.0 |
| **Total volume** | **25.0** | **250.0** |

2. **Add 25 µl of diluted sequencing primer to each well of the PyroMark Q24 Plate according to the run setup (see "Protocol 1: Run setup for the PyroMark Q24 system", page 14).**

   Keep one of the PyroMark Q24 Plate Holders (supplied with the PyroMark Q24 Vacuum Workstation) at room temperature (15–25°C) and use it as support when preparing and moving the plate.

3. **Place the PCR plate (or strips) from Protocol 3 and the PyroMark Q24 Plate on the worktable (Figure 2).**

   Ensure that the plate is in the same orientation as when samples were loaded.


**Figure 2. Placement of PCR plate (or strips) and PyroMark Q24 Plate on the vacuum workstation.**

4. **Apply vacuum to the vacuum tool by opening the vacuum switch.**

5. **Carefully lower the filter probes of the vacuum tool into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 seconds. Take care when picking up the vacuum tool.**

   **Note**: Sepharose beads sediment quickly. Capturing of the beads must take place immediately following agitation.

   If more than 1 minute has elapsed since the plate (or strips) was agitated, agitate again for 1 minute before capturing the beads.

6. **Transfer the vacuum tool to the trough containing 40 ml 70% ethanol (Figure 2). Flush the filter probes for 5 seconds.**
7. **Transfer the vacuum tool to the trough containing 40 ml Denaturation Solution (Figure 2). Flush the filter probes for 5 seconds.**
8. **Transfer the tool to the trough containing 50 ml Wash Buffer (Figure 2). Flush the filter probes for 10 seconds.**
9. **Raise the vacuum tool up and back, beyond 90° vertical, for 5 seconds to drain liquid from the filter probes (Figure 3).**

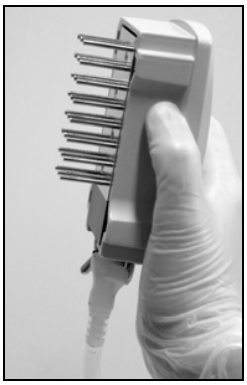


**Figure 3. Illustration of the vacuum tool raised to beyond 90° vertical.**

10. **While the vacuum tool is held over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).**
11. **Release the beads into the PyroMark Q24 Plate by lowering the filter probes into the diluted sequencing primer and moving the tool gently from side to side.**

    Take care not to damage the surface of the PyroMark Q24 Plate by scratching it with the filter probes.

12. **Transfer the vacuum tool to the trough containing high-purity water (Figure 2) and agitate the tool for 10 seconds.**
13. **Wash the filter probes by lowering the probes into high-purity water (Figure 2) and applying vacuum. Flush the probes with 70 ml high-purity water.**
14. **Raise the tool up and back, beyond 90° vertical, for 5 seconds to drain liquid from the filter probes (Figure 3).**

15. **Close the vacuum switch on the tool (Off), and place the tool in the Parking (P) position.**
16. **Turn off the vacuum pump.**

    **Note**: At the end of a working day, liquid waste and remaining solutions should be discarded and the PyroMark Q24 Vacuum Workstation should be checked for dust and spillage (see Appendix B, page 43).
17. **Heat the PyroMark Q24 Plate with the samples at 80°C for 2 minutes using the prewarmed PyroMark Q24 Plate Holder.**
18. **Remove the PyroMark Q24 Plate from the hot plate holder and place it on a second PyroMark Q24 Plate Holder that was kept at room temperature (15–25°C) to let the samples cool to room temperature for 10–15 minutes.**
19. **Proceed with "Protocol 5: Running the PyroMark Q24", page 25.**

## Protocol 5: Running the PyroMark Q24

This protocol describes the preparation and loading of PyroMark Gold Q24 reagents into the PyroMark Q24 Cartridge, and starting and finishing a run on the PyroMark Q24. For a detailed description on how to set up a run, see the *PyroMark Q24 User Manual*.

### Important point before starting

- The Pre Run information report, found in the "Tools" menu at run setup (see "Protocol 1: Run setup for the PyroMark Q24 system", page 14), provides information about the volume of nucleotides, enzyme, and substrate buffer needed for a specific run.

### Things to do before starting

- Switch on the PyroMark Q24. The power switch is located at the rear of the instrument.

### Procedure

1. **Dissolve each of the freeze-dried enzyme and substrate mixtures in 620 µl each of water ($H_2O$, supplied).**
2. **Mix by swirling the vial gently.**

   Do not vortex!

   In order to ensure the mixture is fully dissolved, leave it at room temperature (15–25°C) for 5–10 minutes. Make sure that the solution is not turbid before filling the PyroMark Q24 Cartridge. If the reagents are not to be used immediately, place the reagent vials on ice* or in a refrigerator.
3. **Allow the reagents and the PyroMark Q24 Cartridge to reach ambient temperature (20–25°C).**
4. **Place the PyroMark Q24 Cartridge with the label facing you.**
5. **Load the PyroMark Q24 Cartridge with the appropriate volumes of nucleotides, enzyme, and substrate mixes according to Figure 4.**

   Make sure that no air bubbles are transferred from the pipet to the cartridge.

* 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.

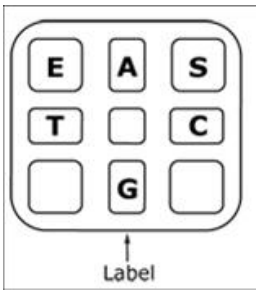



**Figure 4. Illustration of the PyroMark Q24 Cartridge, as seen from above.** The annotations correspond to the label on the reagent vials. Add enzyme mixture (**E**), substrate mixture (**S**), and nucleotides (**A**, **T**, **C**, **G**) according to the volume information given in the Pre Run information report found in the "Tools" menu at run setup.

6. **Open the cartridge gate and insert the filled reagent cartridge with the label facing out. Push the cartridge in fully and then push it down.**
7. **Ensure the line is visible in front of the cartridge and close the gate.**
8. **Open the plate-holding frame and place the plate on the heating block.**
9. **Close the plate-holding frame and the instrument lid.**
10. **Insert the USB stick (containing the run file) into the USB port at the front of the instrument.**

    Do not remove the USB stick before the run is finished.
11. **Select "Run" in the main menu (using the ▲ and ▼ screen buttons) and press "OK".**
12. **Select the run file using the ▲ and ▼ screen buttons.**

    To view the contents of a folder, select the folder and press "Select". To go back to the previous view, press "Back".
13. **When the run file is selected, press "Select" to start the run.**
14. **When the run is finished and the instrument confirms that the run file has been saved to the USB stick, press "Close".**
15. **Remove the USB stick.**
16. **Open the instrument lid.**
17. **Open the cartridge gate and remove the reagent cartridge by lifting it up and pulling it out.**
18. **Close the gate.**
19. **Open the plate-holding frame and remove the plate from the heating block.**
20. **Close the plate-holding frame and the instrument lid.**
21. **Discard the plate and clean the cartridge, as per the instructions in the product sheet supplied with the cartridge.**
22. **Analyze the run according to "Protocol 6: Analysis of a PyroMark Q24 run", page 27.**

## Protocol 6: Analysis of a PyroMark Q24 run

This protocol describes the methylation analysis of a completed *therascreen* MGMT run using PyroMark Q24 Software.

### Procedure

1. **Insert the USB stick (containing the processed run file) into the computer's USB port.**
2. **Move the run file from the USB stick to the desired location on the computer using Windows Explorer.**
3. **Open the run file in CpG mode of the PyroMark Q24 Software either by selecting "Open" in the "File" menu or by double-clicking the file ( ) in the shortcut browser.**
4. **To analyze the run and get an overview of the results, click one of the Analyze buttons.**

    Analyze all wells.

    Analyze the selected well.

   The analysis results (methylation frequencies) and quality assessment are displayed above the variable position in the Pyrogram® trace. For more details on how to analyze a run, see the *PyroMark Q24 User Manual*.

5. **To generate a report, select "CpG Full Report" or "CpG Analysis Results" in the "Reports" menu.**

   **Note**: For reliable results, we recommend single peak heights above 30 RLU. Set 30 RLU as the "required peak height for passed quality" in assay setup (see Appendix A, page 42, and the *PyroMark Q24 User Manual*).

   **Note**: The CpG Analysis Results report should be used for documentation and interpretation of methylation quantification. The numbers shown in the Pyrogram are rounded and do not show the exact quantification.

   **Note**: The Pyrogram should always be compared to the histogram which can be displayed by right-clicking in the Pyrogram window. The measured peaks should match the height of the histogram bars.

## Interpretation of Results

It is recommended that a DNA sample derived from a healthy blood donor is included in every run for comparison.

The bisulfite conversion control (marked by a yellow bar in the Pyrogram window) indicates the completeness of bisulfite conversion. A signal in the bisulfite conversion control might indicate an incomplete bisulfite conversion, which can result in biased methylation quantification and will generate a warning.

The limit of blank (LOB) values represent methylation frequencies obtained from healthy blood donor samples with a probability of 95% (see Table 8 and "Performance Characteristics", page 33).

**Table 8. LOB determined for specific methylation sites using samples from healthy blood donors**

| Position | LOB (% units) |
|---|---|
| CpG site 1 | 1.5 |
| CpG site 2 | 1.8 |
| CpG site 3 | 3.2 |
| CpG site 4 | 3.4 |
| Mean of CpG site 1 to 4 | 2.1 |

**Note**: These values were based on runs where the signal was over 30 relative light units (RLU), as routinely obtained from 10 ng of DNA isolated from blood (measured before bisulfite conversion). We recommend that the method performance is confirmed in the laboratory.

## Representative results

Representative Pyrogram results are shown in Figures 5–7.

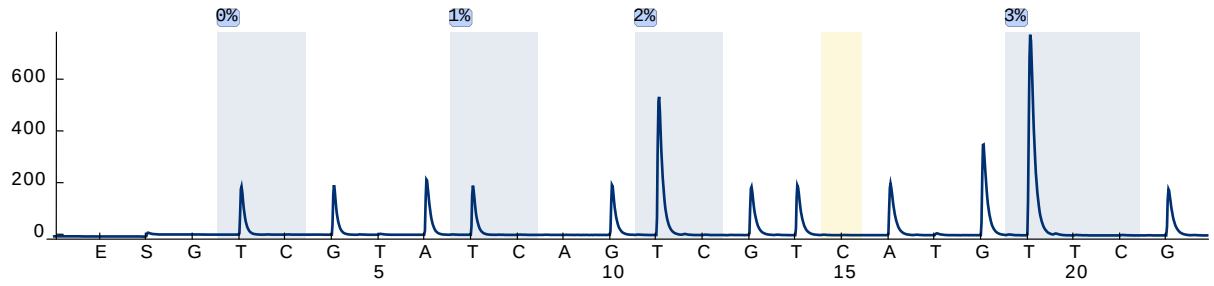



**Figure 5. Pyrogram trace obtained after analysis of unmethylated bisulfite converted DNA from a healthy blood donor sample.** The bar at dispensation 15 represents the control for completion of bisulfite conversion.

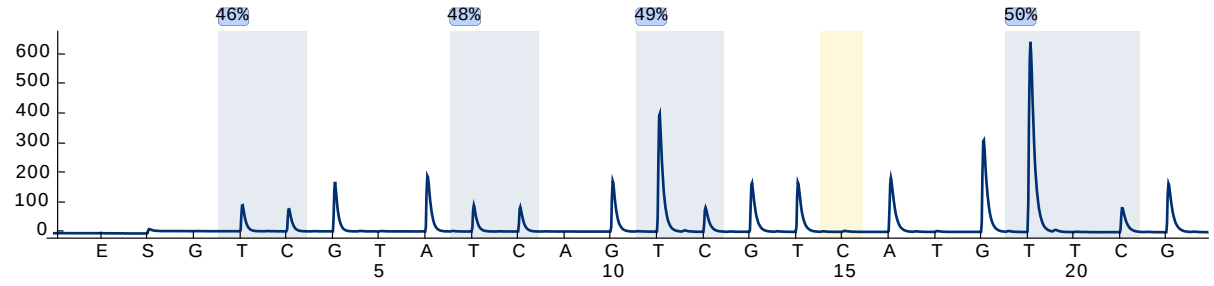



**Figure 6. Pyrogram trace obtained after analysis of methylated bisulfite converted DNA.** The bar at dispensation 15 represents the control for completion of bisulfite conversion.

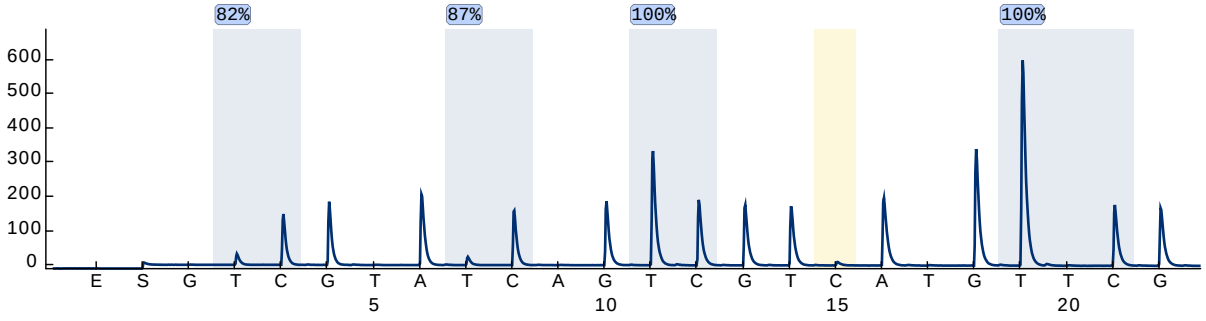



**Figure 7. Pyrogram trace obtained after analysis of highly methylated bisulfite converted DNA (Methylated Control DNA, provided).** The bar at dispensation 15 represents the control for completion of bisulfite conversion.

## Troubleshooting guide

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

**Note**: Refer to the *PyroMark Q24 User Manual* for general troubleshooting of the instrument.

| | Comments and suggestions |
|---|---|
| **Signals in the no template control (negative control)** | |
| a) Cross-talk between wells | Signal from one well is detected in a neighboring well. Avoid placing samples with high signal intensities next to "no template control" wells. |
| b) PCR contamination | Use sterile pipet tips with filters. Store and extract materials such as specimens, controls, and amplicons separately from PCR reagents. |
| **Poor or unexpected sequence** | |
| a) Low quality of genomic DNA | Low-quality genomic DNA can cause the PCR to fail. Analyze PCR samples using an electrophoretic technique (for example, the QIAxcel® System or agarose-gel electrophoresis). |

### "Check" or "failed" result

| | |
|---|---|
| a) Low peak height | Handling errors in PCR setup or sample preparation prior to Pyrosequencing can result in low peaks.<br><br>It is important that the samples are completely taken up by the vacuum tool. Take care that the vacuum tool is lowered slowly into the samples and that the geometry of the PCR plate or strips used for immobilization allows complete take up of the samples.<br><br>Perform the function test for filter probes as described in the *PyroMark Q24 User Manual* on a regular basis and exchange filter probes when indicated.<br><br>In case of a "Check" warning, carefully compare the Pyrogram to the histogram which can be displayed by right-clicking in the Pyrogram window. If the measured peaks match the height of the histogram bars, the result is valid. Otherwise, it is recommended to rerun the sample. |
| b) Warning message "Uncertain/Failed bisulfite conversion at dispensation: 15" appears | Ensure that the value for "Allowed percentage for passed quality" and "Allowed percentage for check quality" are set to 7.0 and 10.0, respectively.<br><br>**Note**: In case of a "Check" or "Failed" quality assessment, the bisulfite conversion was not complete, which can affect the methylation quantification.<br><br>It is recommended to use the EpiTect Bisulfite Kit (cat. no. 59104), EpiTect Plus FFPE Bisulfite Kit (cat. no. 59144), or EpiTect Plus DNA Bisulfite Kit (cat. no. 59124) from QIAGEN, and to strictly follow the protocol for conversion. |

### High background

| | |
|---|---|
| a) Incorrect storage of nucleotides | Store nucleotides at 2–8°C. Storage at –15 to –25°C can cause an increase in the background. |

| | | |
|---|---|---|
| b) | Short cooling time of samples prior to Pyrosequencing analysis | Keep the samples on a PyroMark Q24 Plate Holder at room temperature for 10–15 minutes. Do not shorten the cooling time. |
| c) | Contamination of cartridge | Carefully clean the cartridge as described in the product sheet. Store the cartridge protected from light and dust. |

**No signals in positive controls**

| | | |
|---|---|---|
| a) | Insufficient enzyme or substrate mix for all wells | Make sure to fill the PyroMark Q24 Cartridge according to the "Pre Run Information" in the "Tools" menu. |
| b) | Reagents incorrectly stored or diluted | Prepare the *therascreen* reagents according to the instructions in "Protocol 5: Running the PyroMark Q24", page 25. |
| c) | PCR or sample preparation failure | Handling errors in PCR setup, programming of the PCR cycler, or sample preparation prior to Pyrosequencing analysis can result in no signal. Perform the function test for filter probes as described in the *PyroMark Q24 User Manual* and exchange filter probes when needed. Repeat the PCR and Pyrosequencing analysis. |

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the *therascreen* MGMT Pyro Kit is tested against predetermined specifications to ensure consistent product quality.

# Limitations

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies.

# Performance Characteristics

## Limit of blank

The limit of blank (LOB, Table 9) has been determined for the four CpG sites analyzed by the *therascreen* MGMT Pyro Kit using DNA samples from healthy blood donors according to the recommendations in the Clinical and Laboratory Standards Institute (CLSI) Guideline EP17-A "Protocol for determination of limits of detection and limits of quantitation; approved guideline". The $\alpha$- and $\beta$-errors (false positive and false negative, respectively) were set to 5%.

The LOB values represent methylation frequencies obtained from healthy blood donor samples with a probability of 95%.

**Table 9. LOB determined for specific methylation sites using samples from healthy blood donors**

| Position | LOB (% units) |
|---|---|
| CpG site 1 | 1.5 |
| CpG site 2 | 1.8 |
| CpG site 3 | 3.2 |
| CpG site 4 | 3.4 |
| Mean of CpG site 1 to 4 | 2.1 |

**Note**: It is recommended that the method performance is confirmed in the laboratory.

## Linearity

Linearity was determined using mixtures of unmethylated and methylated bisulfite converted genomic DNA from the EpiTect PCR Control DNA set (cat. no. 59104) and in parallel using mixtures of plasmids carrying the respective bisulfite converted sequence of an unmethylated or methylated sample (i.e., carrying C and T nucleotides in CpG sites, respectively). The genomic DNAs and plasmids, respectively, were mixed in proportions to give twelve levels of methylation (0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%). Each mixture was analyzed with three different lots of the *therascreen* MGMT Pyro Kit in three Pyrosequencing runs with three replicates each.

The results (n=9 for each mutation level) were analyzed according to the CLSI Guideline EP6-A "Evaluation of the linearity of quantitative measurement procedures: a statistical approach; approved guideline" using the Analyse-it® Software v2.21 (Analyse-it Software, Ltd., UK) and are shown in Figure 8 and 9 for the mean methylation of CpG site 1 to 4 using genomic or plasmid DNA as template, respectively.

The results were linear with an allowable nonlinearity of 5 % units in the tested range of 0 to 100% methylation level for each individual methylation site and for the mean of the four methylation sites.

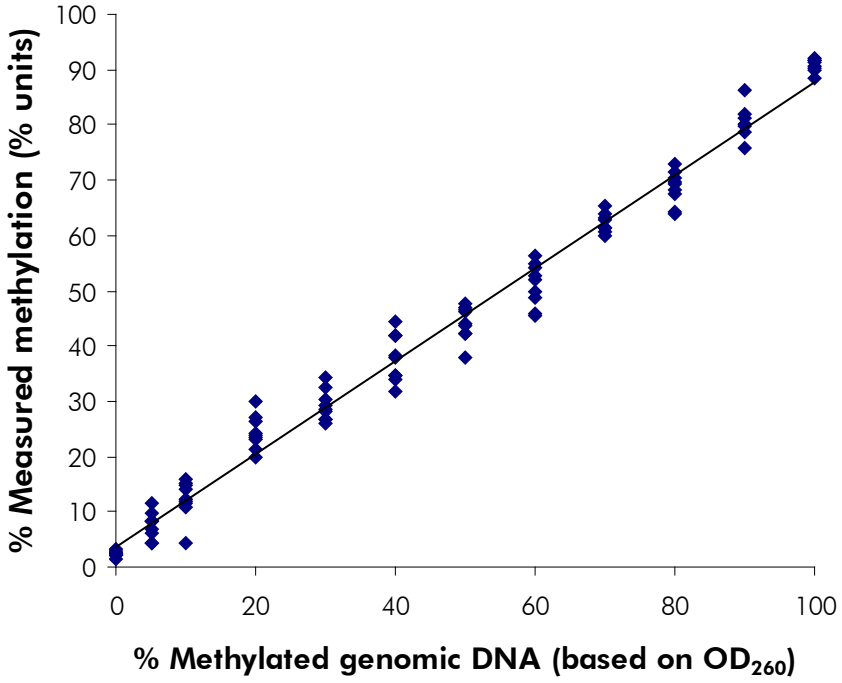



**Figure 8. Linearity of the mean methylation of CpG site 1 to 4 using mixtures of Epitect control DNA.**

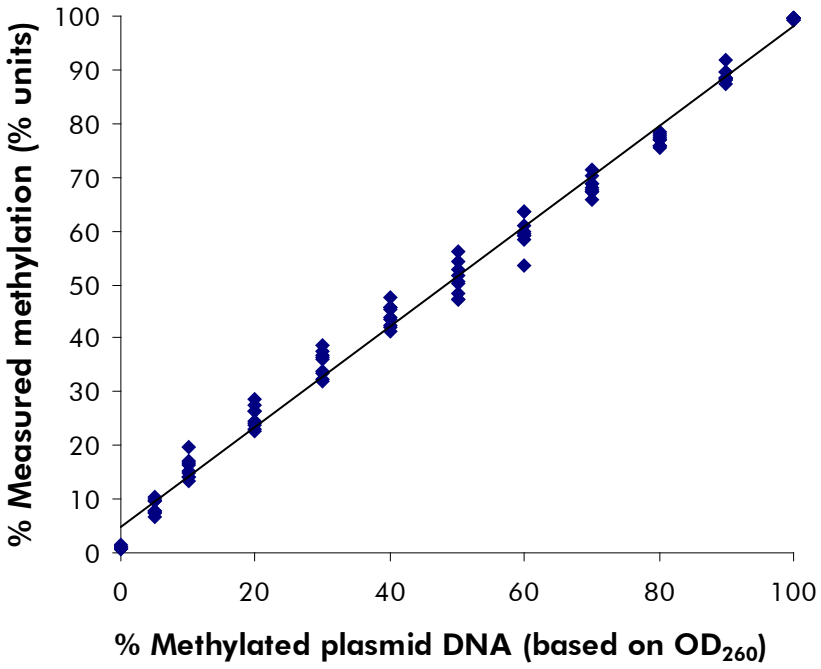



**Figure 9. Linearity of the mean methylation of CpG site 1 to 4 using mixtures of plasmid DNA.**

## Precision

The precision data allows the determination of the total variability of the assay and was obtained at three different levels by analysis of the above mentioned mixtures of genomic and plasmid DNA with three replicates each.

Repeatability (intra-assay and inter-batch variability) was calculated based on the data for determination of linearity (three runs on the same day using varying lots of the *therascreen* MGMT Pyro Kit). Intermediate precision (intra-laboratory variability) was determined in three runs within one laboratory on three different days with varying operators, PyroMark Q24 instruments, and lots of the *therascreen* MGMT Pyro Kit. Reproducibility (inter-laboratory variability) was calculated from two runs each in an internal and external laboratory and using varying lots of the *therascreen* MGMT Pyro Kit.

Precision estimates are expressed as standard deviation of the measured mean methylation frequencies of CpG site 1 to 4 in % units (Tables 10 and 11). The repeatability, intermediate precision, and reproducibility using mixtures of genomic DNA was within 0.5–4.3, 0.4–4.0, and 0.4–4.4 % units, respectively, in the measured range of 0–100% methylation level. Similar results were obtained using mixtures of plasmid DNA (see Table 11).

**Table 10. Precision for the mean methylation of CpG site 1 to 4 using mixtures of EpiTect control DNA***

| % Methylated EpiTect control DNA† | Repeatability | | Intermediate precision | | Reproducibility | |
|---|---|---|---|---|---|---|
| | Mean | SD‡ | Mean | SD | Mean | SD |
| 0 | 2.4 | 0.5 | 2.2 | 0.4 | 2.6 | 0.7 |
| 5 | 7.1 | 2.7 | 7.7 | 2.5 | 9.3 | 3.9 |
| 10 | 12.8 | 2.2 | 12.9 | 2.3 | 15.3 | 3.3 |
| 20 | 23.7 | 2.3 | 23.6 | 2.2 | 24.2 | 2.6 |
| 30 | 29.8 | 2.6 | 31.0 | 2.6 | 30.4 | 3.0 |
| 40 | 36.7 | 3.3 | 37.0 | 3.6 | 38.1 | 3.7 |
| 50 | 44.1 | 2.9 | 44.8 | 3.6 | 44.2 | 2.7 |
| 60 | 51.3 | 3.6 | 52.4 | 3.5 | 51.2 | 3.3 |
| 70 | 62.3 | 1.9 | 62.8 | 2.1 | 61.2 | 2.9 |
| 80 | 68.6 | 3.1 | 69.4 | 3.1 | 66.9 | 3.4 |
| 90 | 80.6 | 3.3 | 79.5 | 2.2 | 77.0 | 4.3 |
| 100 | 90.8 | 1.2 | 91.7 | 2.1 | 90.0 | 1.9 |

* All values are given as % units.

† Based on $OD_{260}$ measurement.

‡ SD: standard deviation (n=9 for repeatability and intermediate precision, n=12 for reproducibility).

**Table 11. Precision for the mean methylation of CpG site 1 to 4 using mixtures of plasmid DNA***

| Plasmid DNA mixture (%)† | Repeatability | | Intermediate precision | | Reproducibility | |
|---|---|---|---|---|---|---|
| | Mean | SD‡ | Mean | SD | Mean | SD |
| 0 | 1.1 | 0.2 | 1.0 | 0.1 | 1.1 | 0.3 |
| 5 | 8.6 | 1.4 | 8.3 | 1.1 | 10.2 | 3.0 |
| 10 | 15.7 | 1.9 | 15.1 | 2.8 | 18.8 | 3.2 |
| 20 | 25.3 | 2.1 | 25.5 | 3.1 | 28.4 | 3.6 |
| 30 | 35.2 | 2.3 | 34.3 | 3.2 | 36.2 | 2.5 |
| 40 | 44.1 | 2.0 | 43.7 | 3.3 | 42.8 | 2.4 |
| 50 | 50.3 | 3.2 | 51.8 | 2.9 | 52.1 | 2.5 |
| 60 | 60.2 | 2.2 | 60.9 | 2.8 | 59.3 | 2.3 |
| 70 | 68.4 | 1.7 | 68.7 | 1.5 | 66.9 | 2.7 |
| 80 | 76.9 | 1.1 | 77.4 | 0.8 | 75.7 | 2.1 |
| 90 | 88.9 | 1.3 | 88.8 | 1.7 | 85.1 | 4.6 |
| 100 | 99.5 | 0.1 | 99.5 | 0.2 | 99.0 | 0.8 |

* All values are given as % units.

† Based on $OD_{260}$ measurement. The 0–100% values indicate the proportion of plasmid carrying C nucleotides in CpG sites (representing methylated C nucleotides) in a mixture with plasmid carrying T nucleotides in CpG sites (representing unmethylated C nucleotides).

‡ SD: standard deviation (n=9 for repeatability and intermediate precision, n=12 for reproducibility).

## Diagnostic evaluation

The *therascreen* MGMT Pyro Kit was evaluated in comparison with Sanger sequencing. DNA was extracted from 100 formalin-fixed paraffin-embedded (FFPE) tumor samples from glioblastoma and analyzed for methylation in the four CpG sites analyzed by the *therascreen* MGMT Pyro Kit.

DNA was isolated using the QIAamp DNA FFPE Tissue Kit and bisulfite converted using the Epitect Bisulfite Kit. Pyrosequencing analysis was carried out with the *therascreen* MGMT Pyro Kit on the PyroMark Q24 and Sanger sequencing on the ABI™ 3130 Genetic Analyzer.

Of 100 samples analyzed by Sanger sequencing, the methylation status could be determined in 49 samples, while with the *therascreen* MGMT Pyro Kit it was possible to determine the methylation level in all samples. Mean methylation levels between 1 and 74 % units were detected in the 100 samples by Pyrosequencing analysis (Figure 10). The distribution of methylation levels for individual sites is shown in Figure 11.

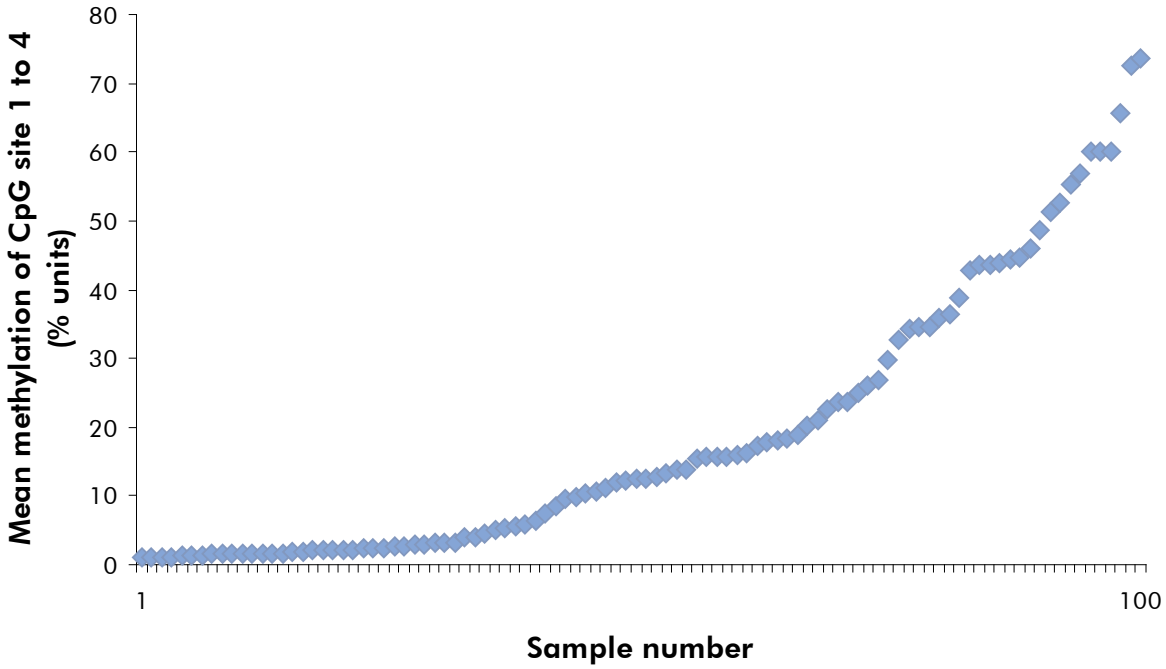



**Figure 10. Mean methylation of CpG site 1 to 4 obtained for 100 glioblastoma samples using the *therascreen* MGMT Pyro Kit.** Samples are sorted by ascending methylation level.

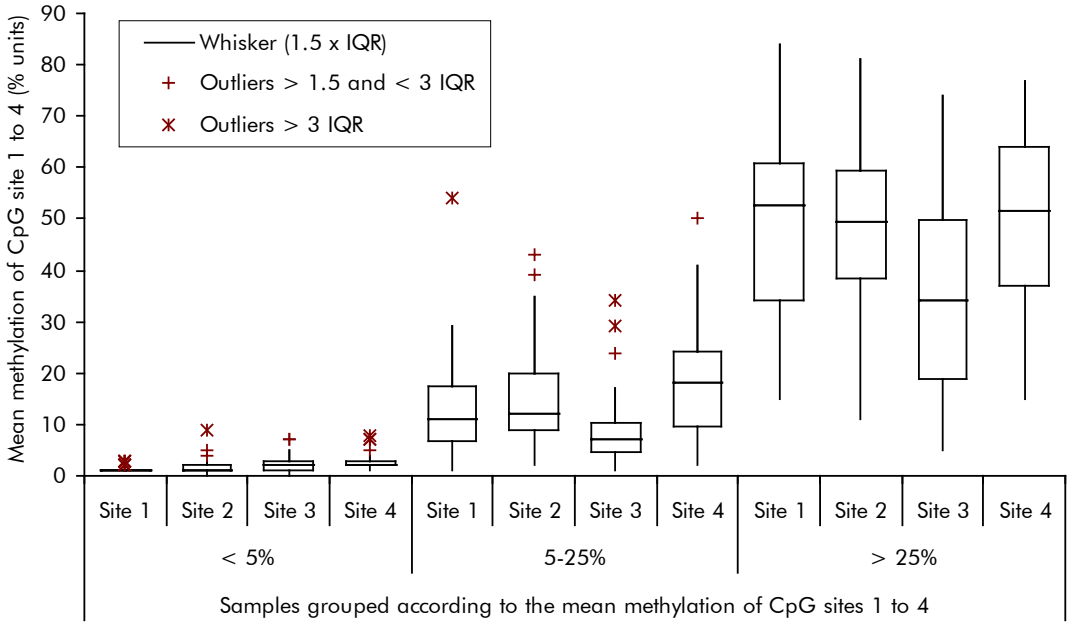



**Figure 11. Distribution of individual CpG site methylation in 100 glioblastoma samples using the *therascreen* MGMT Pyro Kit.** The samples are grouped by the mean methylation of CpG sites 1 to 4. The boxes represent the upper and lower quartiles (25th and 75th percentiles) as separated by the median (50th percentile, shown as horizontal line). Data falling outside this range are shown as Whiskers and Outliers as indicated in the box plot legend. IQR: Interquartile range.

For method comparison, an unmethylated or methylated status was assigned to the Pyrosequencing analysis results using 5 % units mean methylation of CpG site 1 to 4 as a cutoff, while Sanger sequencing results were manually allocated to unmethylated or methylated status.

Thirty-two samples were detected as methylated by Sanger sequencing. In all cases the methylation status could be reproduced with the *therascreen* MGMT Pyro Kit. Two additional samples were reported as methylated by Pyrosequencing, while methylation was not detected for those by Sanger sequencing. Out of 19 unmethylated samples as detected by Sanger sequencing, the same result was reported for 17 samples using the *therascreen* MGMT Pyro Kit. The results are illustrated in Table 12.

Excluding samples that failed in Sanger sequencing analysis, the *therascreen* MGMT Pyro Kit and Sanger sequencing showed 96% concordance in results (Table 12).

**Table 12. Results of methylated analysis in CpG site 1 to 4 for the analyzed glioblastoma samples**

| *therascreen* MGMT Pyro Kit | Sanger sequencing | | | |
|---|---|---|---|---|
| | **Unmethylated** | **Methylated** | **Unknown** | **Total** |
| **Unmethylated** | 17 | 0 | 18 | **35** |
| **Methylated** | 2 | 32 | 31 | **65** |
| **Unknown** | 0 | 0 | 0 | **0** |
| **Total** | **19** | **32** | **49** | **100** |

**Note**: In all runs used for determination of performance characteristics, the signal was over 30 RLU, as routinely obtained from 10 ng of DNA isolated from blood (measured before bisulfite conversion).

# 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.

## Symbols

Contains reagents sufficient for <N> tests


Use by

In vitro diagnostic medical device

Catalog number

Lot number

Material number

Components

Contains

Number

Sodium hydroxide

Global Trade Item Number


Temperature limitation


Manufacturer

Consult instructions for use

## Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

# Appendix A: Setting Up the MGMT Assay

Before running the MGMT assay for the first time, the assay file must be set up. Set up the MGMT assay by using the PyroMark Q24 Software, as described below.

**Procedure**

1. **Click in the toolbar and select "New CpG Assay".**
2. **Type the sequence in "Sequence to Analyze":**
   **YGAYGTTYGTAGGTTTTYGT**
3. **Manually enter the following "Dispensation Order":**
   **GTCGTATCAGTCGTCATGTTCG**
4. **Click the "Analysis Parameters" tab and increase "Peak Height Threshold - Required peak height for Passed quality:" to *30*.**
5. **In the "Analysis Parameters" tab, set the "Allowed percentage for passed quality" and "Allowed percentage for check quality" to *7.0* and *10.0*, respectively.**
6. **Click in the toolbar, and save the assay as "MGMT".**

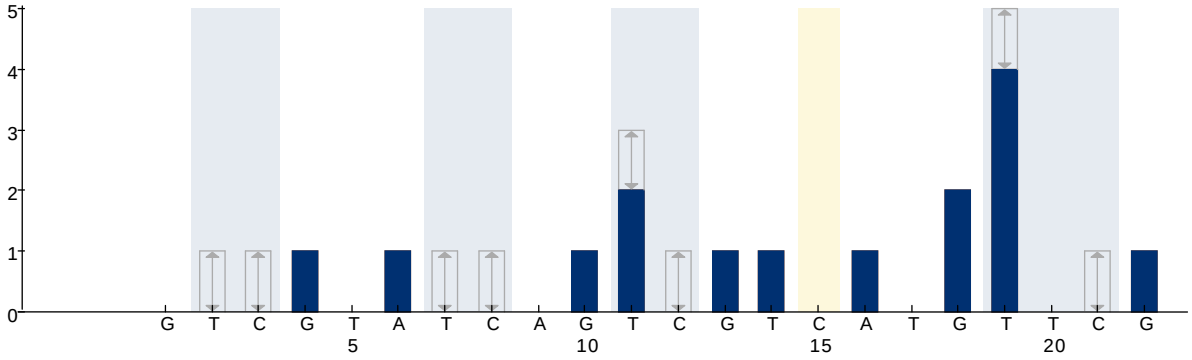



**Figure 12. Histogram for the MGMT assay.** The bar at dispensation 15 indicates the control for completion of bisulfite conversion.

# Appendix B: Emptying the Waste Container and Troughs

| WARNING | Hazardous chemicals |
|---|---|
|  | The Denaturation Solution used with the vacuum workstation contains sodium hydroxide, which is irritating to eyes and skin.<br>Always wear safety glasses, gloves, and a lab coat.<br>The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents.<br>Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws. |

* OSHA: Occupational Safety and Health Administration (United States of America)

† ACGIH: American Conference of Government Industrial Hygienists (United States of America)

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom)

Be sure to observe federal, state, and local environmental regulations for the disposal of laboratory waste.

### Important point before starting

- This protocol requires high-purity water.

### Procedure

**B1. Ensure that no vacuum is applied to the vacuum tool. Make sure that the vacuum is closed (Off) and the vacuum pump is switched off.**

**B2. Discard any solutions left in the troughs.**

**B3. Rinse the troughs with high-purity water, or replace them if necessary.**

**B4. Empty the waste container.**

**B5. The cap can be removed without disconnecting the tubing.**

**B6. If the vacuum workstation must be cleaned (for example, due to dust or spillage), follow the instructions in the *PyroMark Q24 User Manual*.**

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| *therascreen* MGMT Pyro Kit (48) | For 48 reactions on PyroMark Q24 Systems: Seq Primers, PCR Primers, Methylated Control DNA, PyroMark PCR Master Mix, CoralLoad Concentrate, PyroMark Binding Buffer, PyroMark Annealing Buffer, PyroMark Denaturation Solution, PyroMark Wash Buffer, Enzyme Mixture, Substrate Mixture, dATPαS, dCTP, dGTP, dTTP, and $H_2O$ | 971061 |
| **Accessories** | | |
| PyroMark Q24 Plate (100) | 24-well sequencing reaction plate | 979301 |
| PyroMark Q24 Cartridge (3) | Cartridges for dispensing nucleotides and reagents | 979302 |
| PyroMark Vacuum Prep Filter Probe (100) | Reusable filter probes for PyroMark Vacuum Workstation Q96 and Q24 | 979010 |
| PyroMark Control Oligo | For installation check of system | 979303 |
| PyroMark Q24 Validation Oligo | For performance confirmation of system | 979304 |
| **Related products** | | |
| PyroMark Q24 MDx | Sequence-based detection platform for Pyrosequencing of 24 samples in parallel | 9001513 |
| PyroMark Q24 | Sequence-based detection platform for Pyrosequencing of 24 samples in parallel | 9001514 |

| Product | Contents | Cat. no. |
|---|---|---|
| PyroMark Q24 MDx Vacuum Workstation | Vacuum Workstation (220 V) for preparing 24 samples in parallel, from PCR product to single-stranded template | 9001517*<br>9001515† |
| PyroMark Q24 Vacuum Workstation | Vacuum Workstation (220 V) for preparing 24 samples in parallel, from PCR product to single-stranded template | 9001518 |
| PyroMark Q24 MDx Software | Application software | 9019063 |
| PyroMark Q24 Software | Analysis software | 9019062 |
| QIAamp DNA FFPE Tissue Kit (50) | For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Buffers, Collection Tubes (2 ml) | 56404 |
| EZ1 DNA Tissue Kit (48) | For 48 preps: Reagent Cartridges (Tissue), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K | 953034 |
| QIAamp DSP DNA Blood Mini Kit | For 50 preps: QIAamp Mini Spin Columns, Buffers, Reagents, Tubes, VacConnectors | 61104 |
| EpiTect Bisulfite Kit | For 48 preps: EpiTect Bisulfite Spin Columns, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers | 59104 |
| EpiTect Plus FFPE Bisulfite Kit | For 48 preps: MinElute DNA spin columns, Bisulfite mix, DNA Protect Buffer, Carrier RNA, Buffers, Deparaffinization Solution, Lysis Buffer FTB | 59144 |

* UK only.

† Rest of world.

| Product | Contents | Cat. no. |
|---|---|---|
| EpiTect Plus DNA Bisulfite Kit | For 48 prep: MinElute DNA spin columns, Bisulfite mix, DNA Protect Buffer, Carrier RNA, Buffers | 59124 |
| EpiTect PCR Control DNA Set (100) | Human control DNA set (containing both bisulfite converted methylated and unmethylated DNA and unconverted unmethylated DNA) for 100 control PCRs | 59695 |

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