April 2026

MGMT Pyro® Kit Handbook

For quantification of methylation level of the *MGMT* gene using PyroMark® Q24, PyroMark Q24 Advanced, and PyroMark Q48 Autoprep

Sample to Insight

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Kit Contents

MGMT Pyro Kit **Catalog no.** **Number of reactions**	**(48)** **1067482** **48**
PCR Primer Mix MGMT	2 × 24 µL
Seq Primer MGMT	3 × 24 µL
PyroMark PCR Master Mix, 2×	850 µL
CoralLoad® Concentrate, 10×	1.2 mL
H_2O	3 × 1.9 mL
Methylated Control DNA, 10 ng/µL	100 µL

Controls

Methylated Control DNA is included in the kit as a positive control for PCR and sequencing reactions. This control DNA is highly methylated in the regions sequenced using this kit. Include a sample containing Methylated Control DNA for each assay and a negative control (no template DNA) in every PCR setup.

Shipping and Storage

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

Intended Use

The MGMT Pyro 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 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 components.

CAUTION

Always wear safety glasses, gloves, and a lab coat. 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. Venting for fumes and disposal of waste 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).

Quality Control

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

Introduction

Principle and procedure

The MGMT Pyro Kit is used for quantitative measurement of methylation in 4 CpG sites in exon 1 of the human *MGMT* gene (genomic sequence on chromosome 10 from 131,265,519 to 131,265,537: CGACGCCCGCAGGTCCTCG).

Determination of methylation requires bisulfite conversion of DNA to convert unmethylated cytosines to uracils, which are replaced by thymines and can be analyzed by Pyrosequencing. Because methylated cytosines are protected from this conversion, the ratio of cytosines to thymines can be used to determine the methylation level of each CpG site within the target independently.

The procedure comprises four simple steps:

- **Bisulfite conversion of sample DNA.** The EpiTect® Fast Bisulfite Kits (e.g., cat. no. 59802) are recommended for complete bisulfite conversion with minimal DNA degradation.
- **PCR amplification of the region of interest.** The PyroMark PCR Master Mix (included in the kit) is recommended for this amplification, as the provided reagents are optimized for Pyrosequencing analysis.
- **Preparation of single-stranded DNA template.**
- **Sequence analysis of isolated templates using a Pyrosequencing instrument.**

PyroMark MGMT contains forward and reverse PCR primers for amplification of a fragment using bisulfite-treated DNA as template. The reverse primer is biotinylated and enables isolation of the correct template DNA for the sequencing reaction. The included sequencing primer is used in the subsequent Pyrosequencing reaction to quantify the methylation level of the CpG sites and to control for complete conversion of DNA during bisulfite treatment (Figure 1).

Figure 1. Illustration of the *MGMT* assay. The indicated sequence is the analyzed sequence after bisulfite conversion. The asterisk indicates the site for bisulfite conversion control. **FP**, forward PCR primers; **RPB**, reverse PCR primers (B indicates biotinylation); **Seq**, sequencing primers.

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.

- DNA preparation reagents
- EpiTect Fast Bisulfite Conversion Kit (e.g., cat. no. 59802)
- For further information on required equipment and reagents, refer to the user manual of the instrument being used:
    - PyroMark Q24: See Section 5.3, "Sample Preparation," in the *PyroMark Q24 User Manual* (**www.qiagen.com/HB-0240**).
    - PyroMark Q24 Advanced: See Section 5.3, "Sample and Reagent Preparation," in the *PyroMark Q24 Advanced User Manual* (**www.qiagen.com/HB-1341**).
    - PyroMark Q48 Autoprep: See the "Preparing Templates and Reagents" section in the *PyroMark Q48 Autoprep User Manual* (**www.qiagen.com/HB-1971**).

Important Notes

- Create an Assay Setup as described below. This must only be done once, before running the *MGMT* Pyro assay for the first time (see "Protocol: Assay and Run Setup", page 17).
- For bisulfite conversion, use the EpiTect Fast Bisulfite Conversion Kit and follow the instructions in the handbook.
- Ensure that the reactions are thoroughly mixed and prepared and incubated at the recommended temperatures.
- The Sequencing Primer stock solution has a concentration of 10 µM.
    - **PyroMark Q24**: Mix 0.8 µL of undiluted Sequencing Primer stock solution (10 µM) with 24.2 µL Annealing Buffer to prepare 25 µL of a Sequencing Primer solution at a final concentration of 0.32 µM.
    - **PyroMark Q24 Advanced**: Mix 0.75 µL of undiluted Sequencing Primer stock solution (10 µM) with 19.25 µL Advanced Annealing Buffer to prepare 20 µL of a Sequencing Primer solution at a final concentration of 0.375 µM.
    - **PyroMark Q48 Autoprep**: Dilute the Sequencing Primer stock solution (10 µM) to a final concentration of 4 µM by mixing 0.8 µL Sequencing Primer stock solution with 1.2 µL Advanced Annealing Buffer for one reaction.
        - For manual sequencing primer loading, pipet 2 µL of diluted Sequencing Primer (4 µM) into each well.

          Important: Wait until the software prompts you to add the Sequencing Primer (4 µM). Do not pipet the Sequencing Primer (4 µM) into the wells in the beginning of the run. For more information, see *PyroMark Q48 Autoprep User Manual* (**www.qiagen.com/HB-1971**).

- For automatic sequence primer loading, prepare higher volumes of diluted Sequencing Primer (4 µM), as required.

Description of protocols

Before beginning, sample DNA must first be bisulfite converted. This process replaces unmethylated cytosine residues with uracil, while methylated cytosines remain unchanged, giving rise to two different sequences that can be distinguished. The EpiTect Fast Bisulfite Kits (e.g., cat. no. 59802) are recommended for complete conversion with minimal degradation of the treated DNA.

After bisulfite conversion, the next step is to amplify the bisulfite-converted target DNA by PCR, as described in "Protocol: PCR Using the PyroMark PCR Kit", page 14. The *MGMT* Pyro Assay and Run should be set up while the PCR is running, following the instructions in "Protocol: Assay and Run Setup," page Protocol: Assay and Run Setup. After amplification, follow the protocols according to the respective instrument user manual to prepare the sequencing templates for Pyrosequencing analysis:

- *PyroMark Q24 User Manual* (**www.qiagen.com/HB-0240**): See Section 5.3, "Sample preparation," continuing from Section 5.3.3 "Immobilizing the PCR products to beads," and Section 5.4 "Preparation of PyroMark Gold Q24 Reagents."
- *PyroMark Q24 Advanced User Manual* (**www.qiagen.com/HB-1341**): See Section 5.3, "Sample and reagent preparation," continuing from Section 5.3.3, "Immobilizing the PCR products to beads."
- *PyroMark Q48 Autoprep User Manual* (**www.qiagen.com/HB-1971**): See "Preparing templates and reagents," continuing from Section 6.1.5, "Absorber strip".

Finally, perform the Pyrosequencing run and analyze the data according to the relevant instrument user manual:

- *PyroMark Q24 User Manual* (**www.qiagen.com/HB-0240**): See Section 5.5, "Processing a run on the PyroMark Q24 Instrument."
- *PyroMark Q24 Advanced User Manual* (**www.qiagen.com/HB-1341**): See Section 5.4, "Processing a run on the PyroMark Q24 Advanced."
- *PyroMark Q48 Autoprep User Manual* (**www.qiagen.com/HB-1971**): See "Starting a run" and Section 7, "PyroMark Q48 Autoprep Software".

Protocol: PCR Using the PyroMark PCR Kit

This protocol describes the setup and cycling conditions for the amplification of bisulfite-converted DNA using the PyroMark PCR Master Mix (included in kit). The PCR products are subsequently used for quantification of CpG methylation of the *MGMT* gene by Pyrosequencing analysis.

Important points before starting

- See the *PyroMark PCR Kit Handbook* (**www.qiagen.com/HB-3794**) for more detailed information.
- HotStarTaq® DNA Polymerase requires an activation step of 15 min at 95°C (step 5 of the protocol).
- Set up all reaction mixtures in an area separate from areas used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Before opening the tubes containing PCR primers, spin briefly to collect contents at the bottom of the tubes.

Procedure

1. Thaw the PyroMark PCR Master Mix, CoralLoad Concentrate, and primer solutions.

   Important: Mix the solutions before use to avoid localized concentrations of salt.

2. Prepare a reaction mix for each PCR primer set according to Table 1.

Table 1. Preparation of reaction mix for each PCR primer mix

Component of reaction mix	Volume (µL) per reaction
PyroMark PCR Master Mix, 2	12.5
CoralLoad Concentrate, 10×	2.5
PCR Primer *MGMT*	1
RNase-free water	4
Total volume	**20**

3. Gently pipet the reaction mixture up and down to mix thoroughly, and dispense 20 µL into each PCR tube. It is not necessary to keep PCR tubes on ice, as HotStarTaq DNA Polymerase is inactive at room temperature.

4. Add 5 µL bisulfite-converted template DNA (10–50 ng of genomic DNA) to the individual PCR tubes, and mix thoroughly.

   Note: Include a negative control sample (without template DNA) in every PCR setup for at least one assay. In addition, include a sample containing Methylated Control DNA for each assay in every Pyrosequencing run (see "Controls," page 3).

5. Program the thermal cycler according to Table 2.

Table 2. Optimized cycling protocol for PyroMark PCR Master Mix

	Time	Temperature (°C)	Notes
Initial PCR activation step	15 min	95	HotStarTaq DNA Polymerase is activated by this heating step
3-step cycling			
Denaturation	20 s	95	
Annealing	30 s	53	
Extension	20 s	72	
Number of cycles			**42**
Final extension	5 min	72	
Hold	∞	4	

6. Place the PCR tubes in the thermal cycler and start the cycling program.

   Note: After amplification, samples can be stored overnight at 2–8°C. For longer storage store at –30°C to –15°C.

7. Use 10 µL of PCR product for subsequent Pyrosequencing analysis. We recommend checking the PCR product before Pyrosequencing analysis, for example, by rapid analysis on the QIAxcel® system or by agarose gel analysis. See the *PyroMark PCR Kit Handbook* (**www.qiagen.com/HB-3794**) for details. The amplicon length is 105 bp.

8. Proceed to "Protocol: Assay and Run Setup," next page.

Protocol: Assay and Run Setup

These protocols are for setting up the assay parameters and creating a Run Setup for CpG methylation analysis in *MGMT*.

Refer to the section below according to the PyroMark instrument used.

PyroMark Q24

Use default settings in the software for all assay setups if not otherwise stated.

Procedure

1. **Sequence to Analyze** – Set up the *MGMT* assay by selecting **New CpG Assay** in the PyroMark Q24 Software, and enter the following sequence in **Sequence to Analyze**:

   YGAYGTTYGTAGGTTTTYGT

2. **Nucleotide Dispensation Order** – Click **Generate Dispensation Order**. Choose the **T** at dispensation 14 as a control for bisulfite treatment by left-clicking the orange **T**. The following dispensation order should be used:

   GTCGTATCAGTCGTCATGTTCG

   The control for completion of bisulfite treatment is highlighted in gray in the sequence above. It is automatically analyzed by the PyroMark Q24 Software and indicated in yellow (Figure 2).

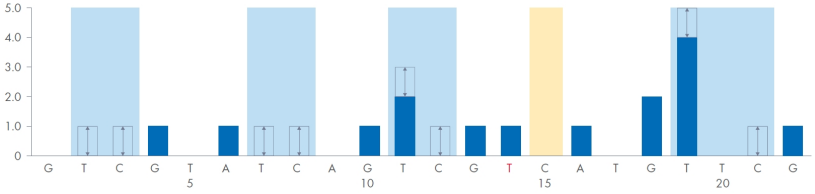

Figure 2. Histogram for the *MGMT* assay.

3. Click the **Analysis Parameters** tab and set **Allowed percentage for passed quality** to 7.0 and **Allowed percentage for check quality** to 10.0.

4. **Run setup** – Create a new run file by selecting **New Run**. Set up the plate by adding the *MGMT* assay to each used well. Proceed with the run setup, preparation of samples, and run according to the instructions in the *PyroMark Q24 User Manual*.

PyroMark Q24 Advanced

Procedure

1. **Sequence to Analyze** – Set up the *MGMT* assay by selecting **New CpG Assay** in the PyroMark Q24 Advanced Software, and enter the following sequence in **Sequence Before Bisulfite Treatment**:

   CGACGCCCGCAGGTCCTCG

   The software automatically generates the following **Sequence to Analyze**:

   YGAYGTTYGTAGGTTTTYGT

2. **Nucleotide Dispensation Order** – Manually enter the following dispensation order:

   GTCGTATCAGTCGTCATGTTCG

The control for completion of bisulfite treatment is highlighted in gray in the sequence above. It is automatically analyzed by the PyroMark Q24 Advanced Software and indicated in orange (Figure 2).

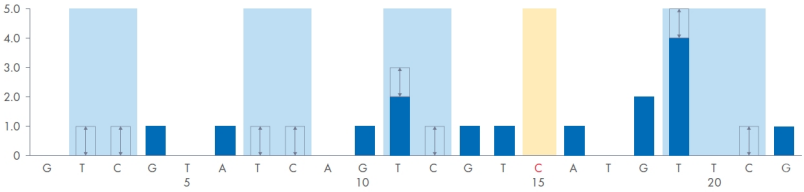

Figure 3. Histogram for the *MGMT* assay.

3. Click the **Analysis Parameters** tab and set **Allowed percentage for passed quality** to 7.0 and **Allowed percentage for check quality** to 10.0.

4. **Run setup** – Create a new run file by selecting **New Run**. Set up the plate by adding the *MGMT* assay to each used well. Proceed with the run setup, preparation of samples, and run according to the instructions in the *PyroMark Q24 Advanced User Manual*.

PyroMark Q48 Autoprep

Procedure

1. **Sequence to Analyze** – Set up the *MGMT* assay by selecting **New CpG Assay** in the PyroMark Q48 Autoprep Software, and enter the following sequence in **Sequence Before Bisulfite Treatment**:

   CGACGCCCGCAGGTCCTCG

   The software automatically generates the following **Sequence to Analyze**:

   YGAYGTTYGTAGGTTTTYGT

2. **Nucleotide Dispensation Order** – Manually enter the following dispensation order:

   GTCGTATCAGTCGTCATGTTCG

   The control for completion of bisulfite treatment is highlighted in gray in the sequence above. It is automatically analyzed by the PyroMark Q48 Autoprep Software and indicated in orange (Figure 2).

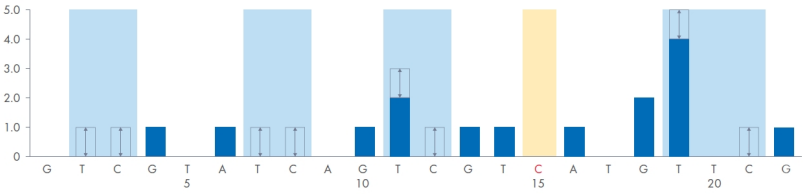

Figure 4. Histogram for the *MGMT* assay.

3. **Run setup** – Create a new run file by selecting **New Run**. Set up the plate by adding the *MGMT* assay to each used well. Proceed with the run setup, preparation of samples, and run according to the instructions in the *PyroMark Q48 Autoprep User Manual*.

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** (for contact information, visit **www.qiagen.com**).

Refer to the user manual for the PyroMark instrument which is used.

- When using PyroMark Q24, see Section 7, "Troubleshooting Guide," in the *PyroMark Q24 User Manual*.
- When using PyroMark Q24 Advanced, see Section 7, "Troubleshooting Guide," in the *PyroMark Q24 Advanced User Manual*.
- When using PyroMark Q48 Autoprep, see Section 9, "Troubleshooting Guide," in the *PyroMark Q48 Autoprep User Manual*.
- For an assay-specific Troubleshooting Guide, see "Troubleshooting Guide," in the *MGMT Pyro Handbook* for PyroMark Q24 (**www.qiagen.com**).

Assay-specific troubleshooting

"Check" or "failed" result	Comments and suggestions
Warning message "Uncertain/Failed bisulfite conversion at dispensation: 15" appears.	Ensure that the values for "Allowed percentage for passed quality" and "Allowed percentage for check quality" are set to 7.0 and 10.0, respectively. **Note**: In cases of a "Check" or "Failed" quality assessment, the bisulfite conversion is not complete, which can affect methylation quantification. QIAGEN recommends using EpiTect Bisulfite Kit (cat. no. 59104), EpiTect Plus FFPE Bisulfite Kit (cat. no. 59144), or EpiTect Plus DNA Bisulfite Kit (cat. no. 59124) and following the protocol for conversion.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **support.qiagen.com** or contact one of the QIAGEN Technical Service Departments or local distributors (visit **support.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
Assays & Controls		
MGMT Pyro Kit	For 48 reactions: Forward primer, Reverse primer, and Sequencing primer for the analysis of *MGMT* methylation using the PyroMark Q24, PyroMark Q24 Advanced, or PyroMark Q48 Autoprep	970061 1067482
PyroMark Control Oligo	For installation check of the system	979203
PCR & Bisulfite Conversion		
PyroMark PCR Kit (200)	PCR Master Mix for PCR reactions optimized for Pyrosequencing analysis	978703
EpiTect Fast DNA Bisulfite Kit (50)	For 50 preps: Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59824
EpiTect Fast FFPE Bisulfite Kit (50)	For 50 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59844
EpiTect Fast LyseAll Bisulfite Kit (50)	For 50 preps: Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59864
EpiTect Fast Bisulfite Kit (10)	For 10 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA, and Buffers	59802
PyroMark Q24		
PyroMark Q24	Instrument, for laboratory use only	9001514
PyroMark Q24 Software	Analysis software, for laboratory use only	9019062
PyroMark Q24 Advanced		
PyroMark Q24 Advanced CpG Reagents (4 × 24)	For 4 × 24 samples for use on the PyroMark Q24 Advanced: Enzyme Mixture, Substrate Mixture, Buffers, and Nucleotides for CpG and long-read sequencing runs	970922
PyroMark Q24 Advanced	Instrument, software, and installation for advanced Pyrosequencing analysis of 24 samples in parallel	9002270

Product	Contents	Cat. no.
PyroMark Q48 Autoprep		
PyroMark Q48 Autoprep	Instrument, software, and pipette	9002470
PyroMark Q48 Discs (50)	50 discs for running PyroMark Q48 Autoprep reactions	974901
PyroMark Q48 Absorber Strips (100)	100 absorber strips for running PyroMark Q48 Autoprep reactions	974912
PyroMark Q48 Autoprep Starter Kit	PyroMark Q48 Magnetic Beads (300), PyroMark Q48 Advanced CpG Reagents (4 × 48), PyroMark Control Oligo, PyroMark Q48 Discs (50) and PyroMark Q48 Absorber Strips (100)	974230
PyroMark Q48 Advanced Reagents (4 × 48)	Reagents for 4 × 48 PyroMark Q48 Autoprep standard reactions	974002
PyroMark Q48 Advanced CpG Reagents (4 × 48)	Reagents for 4 × 48 PyroMark Q48 Autoprep CpG and long-read reactions	974022
PyroMark Q48 Magnetic Beads (300)	Magnetic streptavidin-coated Sepharose beads for running 300 PyroMark Q48 Autoprep reactions	974203

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 Support or your local distributor.

Document Revision History

Date	Description
04/2026	Initial release

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