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PyroMark® Q24 Advanced and PyroMark Q24 Advanced CpG Reagents Handbook

For performing PyroMark Q24 Advanced and long-read Pyrosequencing® reactions

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

We recommend using the PyroMark Q24 Advanced CpG Reagents for long-read Pyrosequencing runs such as de novo sequencing or methylation analyses, which may require larger volumes of nucleotides.

	PyroMark Q24 Advanced Reagents	PyroMark Q24 Advanced CpG Reagents
Catalog no.	**970902**	**970922**
Number of preps	**4 x 24**	**4 x 24**
Enzyme Mixture*	1 vial	1 vial
Substrate Mixture*	1 vial	1 vial
dATPαS	380 µl	1200 µl
dGTP	260µl	660 µl
dCTP	260µl	660 µl
dTTP	260µl	660 µl
PyroMark Q24 Advanced Annealing Buffer	10 ml	10 ml
PyroMark Binding Buffer	10 ml	10 ml

* Mixture supplied as a lyophilized preparation to be reconstituted before use.

Storage

PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents are shipped on dry ice. Store all reagents at 2–8°C. Reconstituted enzyme and substrate mixtures are stable for at least 5 days at 2–8°C. 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 can be frozen and stored in their vials at –30 to –15°C. Frozen reagents should not be subjected to more than 6 freeze–thaw cycles. The Substrate Mixture must be protected from exposure to light. All reagents are stable at the recommended storage conditions until the expiration date.

Important: Do not freeze the nucleotides.

Intended Use

PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding PyroMark Q24 Advanced Reagents, PyroMark Q24 Advanced CpG Reagents, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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).

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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents is tested against predetermined specifications to ensure consistent product quality.

Introduction

PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents are optimized for Pyrosequencing technology. The reagents are designed to generate a Pyrogram® with sharp and distinct peaks and low background, providing optimal conditions for mutation and SNP analyses. PyroMark Q24 Advanced CpG Reagents are recommended for assays with longer sequencing read lengths, such as with CpG methylation analysis or de novo sequencing.

PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents contain all enzymes, substrates, buffers, and nucleotides needed in the Pyrosequencing cascade (Figure 1). When nucleotides are incorporated into the analyzed DNA strand, pyrophosphate is released and converted to ATP. The generation of ATP drives a detectable light signal through a luciferase reaction and this is proportional to the number of nucleotides incorporated.

PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents are intended to be used together with the PyroMark Q24 Cartridge and the PyroMark Q24 Advanced (PyroMark Q24 Instrument running PyroMark Q24 Advanced Software).

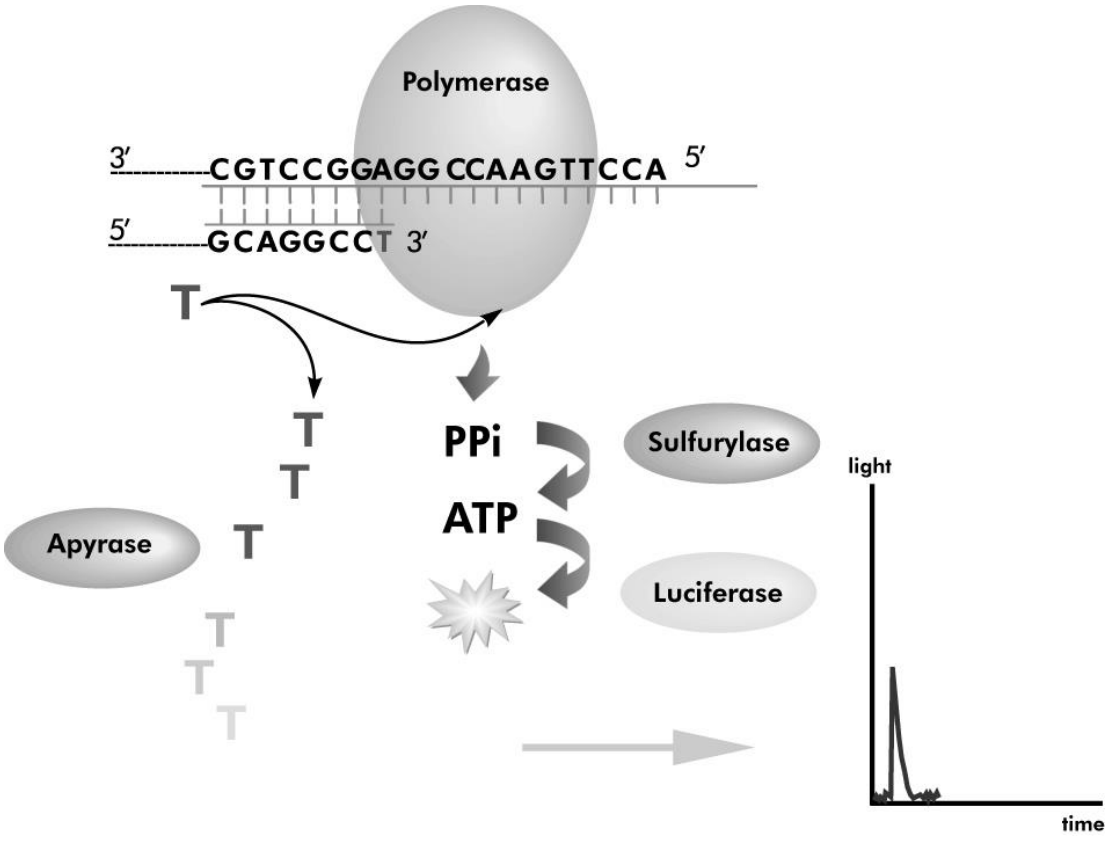

Figure 1. Schematic illustration of the Pyrosequencing cascade. As nucleotides are incorporated into the analyzed DNA strand, pyrophosphate is released and converted to ATP. Generated ATP drives the light reaction detected as a peak on the Pyrogram. Apyrase degrades any unincorporated nucleotides.

Enzyme mixture

The enzyme mixture contains all enzymes that are needed in the Pyrosequencing cascade. These are DNA polymerase for incorporation of nucleotides, ATP sulfurylase for conversion of pyrophosphate to ATP, and luciferase for generation of the light signal. Apyrase is included to degrade ATP and unincorporated nucleotides which switches off the light signal and regenerates the reaction solution. In addition, single-stranded binding protein (SSB) has been added to prevent formation of secondary structures in the DNA template and primers.

Substrate mixture

The substrate mixture consists of adenosine 5' phosphosulfate (APS) needed for generation of ATP, and luciferin, the substrate for luciferase. ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin. This conversion generates visible light in amounts that are proportional to the amount of ATP.

Nucleotides

Nucleotides included in the PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents are dissolved in a well-balanced buffer to prevent degradation of the nucleotides. It should be noted that deoxyadenosine alpha-thio triphosphate (dATPαS) is used as a substitute for the natural deoxyadenosine triphosphate (dATP) since this deoxynucleotide is efficiently used by the DNA polymerase but not recognized by luciferase.

Buffers

Two buffers are included with the PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents. PyroMark Binding Buffer enhances the immobilization of PCR products to the Streptavidin Sepharose® High Performance matrix. Before the sequencing reaction, primers are diluted in PyroMark Q24 Advanced Annealing Buffer.

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 material safety data sheets (MSDSs), available from the product supplier.

- Pipets (adjustable)*
- Sterile pipet tips with filters
- Streptavidin Sepharose High Performance (GE Healthcare, cat. no. 17-5113-01; www.gelifesciences.com)
- PyroMark Q24 Advanced (cat. no. 9002270)
- PyroMark Q24 Plate (cat. no. 979201)
- PyroMark Q24 Cartridge (cat. no. 979202)
- PyroMark Q24 Vacuum Workstation (cat. no. 9001518 [220V], or 9001516 [110V], or 9001519 [100V])
- PyroMark Q24 Control Oligo (cat. no. 979203) for installation check of PyroMark Q24 Advanced system
- PyroMark Q24 Validation Oligo (cat. no. 979204) for performance check of PyroMark Q24 Advanced system
- Plate mixer* for immobilization to beads (see Table 1, page 10)
- Sequencing primer
- Heating block* capable of attaining 80°C
- 24-well PCR plates or strips (see Table 2, page 10)
- High-purity water* (Milli-Q® 18.2 MΩ x cm or equivalent)
- Ethanol (70%)
- PyroMark Wash Buffer
- PyroMark Denaturation Buffer
- Lint-free tissue
- Adhesive foil or strip caps

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

Recommended plate mixers

The orbital plate mixers in Table 1 are recommended for use with the PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents.

Table 1. Recommended plate mixers

Manufacturer	Product	Catalog number
Eppendorf®	Thermomixer comfort (Basic device)	5355 000.011
	Thermoblock for MTP	5363 000.012
	Adapter plate for 96 x 0.2 ml 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)

Recommended 24-well plates

The 24-well plates in Table 2 are recommended for use with the PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents.

Table 2. Recommended 24-well plates

Sample material	Product	Catalog number
ABgene® (Thermo Scientific)	Thermo-Fast PCR Plate	AB-0624
Axygen	24 Well PCR Microplate	PCR-24-C
4titude	FrameStar Break-a-way 96 wells, clear tubes	4ti-1000
Kisker	Quali – PCR Plates without frame	G030

Protocol 1: Immobilization of PCR Product to Sepharose Beads

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

Things to do before starting

- Switch on the PyroMark Q24 Instrument at least 30 minutes before starting a run. The power switch is located at the rear of the instrument.
- Place one PyroMark Q24 Plate Holder on a pre-heated heating block at 80°C.
- PyroMark Wash Buffer is supplied as a 10x concentrate. Before using for the first time, dilute to a 1x working solution by adding 225 ml high-purity water to 25 ml 10x PyroMark Wash Buffer (final volume of 250 ml).
- Prepare the PyroMark Q24 Vacuum Workstation for sample preparation as described in the *PyroMark Q24 Advanced User Manual*.

Procedure

1. **Gently shake the bottle containing Streptavidin Sepharose HP until it is a homogeneous solution.**

   Note: Sepharose beads sediment quickly. Before pipetting, ensure the homogeneity of the bead solution by mixing.

2. **Prepare DNA immobilization reactions according to Table 3, page 12.**

   Note: Sepharose beads sediment quickly. Ensure homogeneity of the reaction mixtures by frequent mixing using a pipet or pulse vortexing. Do not spin down.

   Optional: When the amount of PCR product is the same for each reaction, prepare a master mix for DNA immobilization according to Table 3, page 12, with all components except the PCR product.

Table 3. Reaction setup for DNA immobilization

Component	Volume per sample	
	For lot number 10057037 or higher	For lot numbers lower than 10057037
Streptavidin Sepharose HP*	1 µl	2 µl
PyroMark Binding Buffer	40 µl	40 µl
High-purity water†	Variable†	Variable†
Total volume	**60–75 µl†**	**60–75 µl†**

* Check the lot number of the Streptavidin Sepharose HP. For lot number 10057037 or higher use 1 µl. For lot numbers lower than 10057037, use 2 µl.

† The volume of water depends on the amount of PCR product used. For example, for 15 µl of PCR product, 1 µl of beads, and 40 µl of Binding Buffer, add 24 µl of high-purity water.

3. **Add 60–75 µl of the reaction mixtures or master mix to wells of a 24-well PCR plate as predefined in the run setup in the PyroMark Q24 Advanced software.**

   Note: Sepharose beads sediment quickly. Ensure the homogeneity of the reaction mixtures or master mix by frequent mixing using a pipet or pulse vortexing. Do not spin down.

4. **Add 5–20 µl of biotinylated PCR product to each well containing reaction mixture or master mix as predefined in the run setup.**

   The total volume per well should be 80 µl after addition of the PCR product to the reaction mixture or master mix.

5. **Seal the PCR plate using adhesive foil.**

   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, proceed immediately with "Protocol 2: Loading Reagents into the PyroMark Q24 Cartridge", page 13.

Protocol 2: Loading Reagents into the PyroMark Q24 Cartridge

This protocol describes the loading of PyroMark Q24 Advanced Reagents and PyroMark Q24 Advanced CpG Reagents into the PyroMark Q24 Cartridge, and placing the cartridge in the PyroMark Q24 Instrument. For details, see the *PyroMark Q24 Advanced User Manual*.

Important points before starting

- Dissolve the lyophilized enzyme and substrate mixtures in 660 µl each of high-purity water (Milli-Q 18.2 MΩ x cm, or equivalent filtered through a 0.22 µm filter).

  Note: Mix by swirling the vial gently. Do not vortex! To ensure that 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.

- The Pre Run information report, found in the "Tools" menu at run setup, provides information about the volume of nucleotides, enzyme, and substrate buffer needed for a specific run.

- Use disposable tips without hydrophobic filters for loading the cartridge to permit correct function of the cartridge.

Procedure

1. **Allow the reagents to reach ambient temperature (20–25°C).**
2. **Place the PyroMark Q24 Cartridge with the label facing you.**
3. **Load the PyroMark Q24 Cartridge with the appropriate volumes of nucleotides, enzyme, and substrate mixes (Figure 2). Information on volume is given in the Pre Run information report, found in the "Tools" menu at run setup.**

   Note: If pipetting small volumes of reagents into the cartridge (e.g., below 50 µl per well) make sure that all liquid is collected at the bottom of the cartridge. For example, this can be accomplished by gently tapping the cartridge several times on a smooth work bench.
   Important: Avoid tapping the cartridge too hard or on uneven surfaces, as this can damage the needles. Alternatively, the volume of the liquid used can be increased.

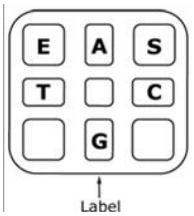

Figure 2. Illustration of the PyroMark Q24 Cartridge as seen from above. The annotations correspond to the label on the reagent vials. Add enzyme mixture (**E**), substrate mixture (**S**), and nucleotides (**A**, **T**, **C**, **G**).

4. **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.**
5. **Ensure the line is visible in front of the cartridge and close the gate.**
6. **Open the plate-holding frame and place the plate on the heating block.**
7. **Close the plate-holding frame and the instrument lid.**
8. **Proceed directly with "Protocol 3: Preparation of Template DNA and Annealing to Primer", page 14.**

Protocol 3: Preparation of Template DNA and Annealing to Primer

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 Advanced.

Important points before starting

- Ensure that the PyroMark Q24 Vacuum Workstation is prepared as described in the *PyroMark Q24 Advanced User Manual*.
- PyroMark Wash Buffer is supplied as a 10x concentrate. Before using for the first time, dilute to a 1x working solution by adding 225 ml high-purity water to 25 ml 10x PyroMark Wash Buffer (final volume of 250 ml).
- Perform the function test for the filter probes as described in the *PyroMark Q24 Advanced User Manual* on a regular basis and exchange filter probes when indicated.

Procedure

1. **Dilute a sufficient amount of each sequencing primer to 0.375 µM with PyroMark Advanced Annealing Buffer.**
2. **Add 20 µl of diluted sequencing primer to each well of the PyroMark Q24 Plate according to the run setup.**
3. **Switch on the vacuum pump of the PyroMark Q24 Vacuum Workstation.**

4. **Place the PCR plate from protocol 1 and the PyroMark Q24 Plate on the vacuum workstation (Figure 1).**

   Inspect the PCR plate and ensure the Sepharose beads are in solution. Ensure that the PCR plate is in the same orientation as when samples were loaded.

Figure 1. Placement of PCR plate and PyroMark Q24 Plate on the vacuum workstation.

5. **Switch on the vacuum and apply vacuum to the tool.**
6. **Slowly lower the filter probes of the vacuum tool into the PCR plate 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 beads must take place immediately following agitation. If more than 1 minute has elapsed since the plate was agitated, agitate again for 1 minute before capturing the beads.

   Inspect the PCR plate for a complete take up of all samples by the vacuum tool.

7. **Transfer the vacuum tool to the trough containing 40 ml 70% ethanol (trough 1; Figure 1). Flush the filter probes for 5 seconds.**
8. **Transfer the vacuum tool to the trough containing 40 ml Denaturation Solution (trough 2; Figure 1). Flush the filter probes for 5 seconds.**
9. **Transfer the vacuum tool to the trough containing 50 ml Wash Buffer (trough 3; Figure 1). Flush the filter probes for 10 seconds.**
10. **Raise the vacuum tool up and back, beyond 90° vertical, for 5 seconds to drain liquid from the filter probes (Figure 2).**

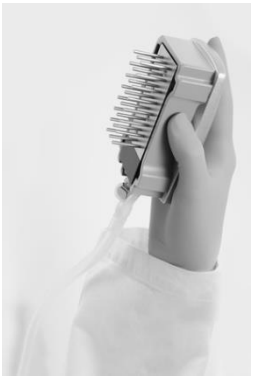

Figure 2. Illustration of the vacuum tool raised to beyond 90° vertical.

11. **While the vacuum tool is held over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).**
12. **Release the beads in 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.
13. **Transfer the vacuum tool to the trough containing high-purity water (trough 4; Figure 1) and agitate it for 10 seconds.**
14. **Wash the filter probes by lowering the probes into high-purity water (trough 5; Figure 1) and applying vacuum. Flush the probes with 70 ml high-purity water.**
15. **Raise the vacuum tool up and back, beyond 90° vertical, for 5 seconds to drain liquid from the filter probes (Figure 2).**
16. **Close the vacuum switch on the tool (Off), and place the vacuum tool in the Parking (P) position.**
17. **Turn off the vacuum pump.**

    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.
18. **Heat the PyroMark Q24 Plate with the samples at 80°C for 5 minutes using the pre-warmed PyroMark Q24 Plate Holder.**
19. **Remove the hot plate holder together with the PyroMark Q24 Plate from the heating block and place the plate immediately on the heating block of the PyroMark Q24 Instrument. Ensure that the plate-holding frame is closed.**

    Note: The time between removing the plate holder from the heating block and placing the PyroMark Q24 plate in the PyroMark Q24 Instrument should not exceed 30 seconds.
20. **Proceed directly with "Protocol 4: Running the PyroMark Q24 Advanced", page 17.**

Protocol 4: Running the PyroMark Q24 Advanced

This protocol describes starting and finishing a run on the PyroMark Q24 Advanced. For a detailed description about how to set up a run, see the *PyroMark Q24 Advanced User Manual*.

Procedure

1. **Make sure that the PyroMark Q24 Cartridge was properly loaded and inserted into the PyroMark Q24 Instrument (see protocol 2).**
2. **Make sure that the PyroMark Q24 Plate containing single-stranded template DNA with primer was properly placed on the heating block of the PyroMark Q24 Instrument (see protocol 3).**
3. **Close the instrument lid.**
4. **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.
5. **Select "Run" in the main menu (using the ▲ and ▼ screen buttons) and press "OK".**
6. **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".
7. **When the run file is selected, press "Select" to start the run.**
8. **When the run is finished and the instrument confirms that the run file has been saved to the USB stick, press "Close".**
9. **Remove the USB stick.**
10. **Open the instrument lid. Open the cartridge gate and remove the reagent cartridge by lifting it up and pulling it out**
11. **Close the gate.**
12. **Open the plate-holding frame and remove the plate from the heating block. Close the plate-holding frame and the instrument lid.**
13. **Discard the plate and clean the cartridge.**

    Cleaning instructions are in the product sheet supplied with the cartridge.
14. **Analyze the run according to the *PyroMark Q24 Advanced 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. 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).

Refer to the *PyroMark Q24 Advanced User Manual* for general troubleshooting of the instrument and the *PyroMark PCR Handbook* for PCR troubleshooting.

	Comments and suggestions
Small or missing peaks in the Pyrogram	
a) Blocked or damaged cartridge needles	Clean the PyroMark Q24 Cartridge and check that it is working correctly. In case of bent needles, discard the PyroMark Q24 Cartridge according to federal, state, and local environmental regulations for disposal of laboratory waste.
b) Reagents incorrectly stored	Be sure to follow the instructions in "*Mixture supplied as a lyophilized preparation to be reconstituted before use. Storage", page 4.
c) Reagents incorrectly dissolved	Be sure to follow the instructions in "Important points before starting", page 13.
d) One or more of the nucleotide compartments in the PyroMark Q24 Cartridge not loaded correctly	Be sure to add enough reagents (select "Pre Run Information" from the "Tools" menu of the PyroMark Q24 Advanced Software). Follow the handbook supplied with the PyroMark Kit used. See "Protocol 2: Loading Reagents into the PyroMark Q24 Cartridge", page 13. .
e) No enzyme or substrate added to the well (noted as a missing presequencing signal and missing peaks in the Pyrogram)	Clean the PyroMark Q24 Cartridge and check that it is working correctly.
f) PyroMark Q24 Cartridge incorrectly inserted	Ensure that the cartridge is inserted correctly.

	Comments and suggestions
High presequencing signal	
Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal)	Change buffers. Only use buffers that are supplied by QIAGEN. Move the pointer over a section of Pyrogram and select with the left mouse button. Use the zoom-in function to check if any peaks have been generated.

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.
PyroMark Q24 Advanced Reagents (4 x 24)	For 4 x 24 samples for use on the PyroMark Q24 Advanced: Enzyme Mixture, Substrate Mixture, Buffers, and Nucleotides	970902
PyroMark Q24 Advanced CpG Reagents (4 x 24)	For 4 x 24 samples for use on the PyroMark Q24 Advanced: Enzyme Mixture, Substrate Mixture, Buffers, and Nucleotides for CpG and long-read sequencing runs	970922
Accessories		
PyroMark Q24 Cartridge (3)	Cartridges for dispensing nucleotides and reagents on the PyroMark Q24 Advanced	979202
Related products		
PyroMark Q24 Advanced	Instrument, software, and installation for advanced Pyrosequencing analysis of 24 samples in parallel	9002270
PyroMark Q24 Vacuum Workstation	Workstation for preparing single-stranded DNA from 24 samples	Varies*
PyroMark Q24 Advanced Software	Application software	9022779
PyroMark Binding Buffer (200 ml)†	For binding of biotinylated PCR product to Streptavidin Sepharose® beads	979006
PyroMark Denaturation Solution (500 ml)‡	For denaturation of double-stranded PCR product into single-stranded template DNA	979007
PyroMark Wash Buffer, concentrate (200 ml)§	For washing of single-stranded DNA	979008

* 9001518 (220V); 9001516 (110V); 9001519 (100V).

† For use with PyroMark Q24, PyroMark Q96 MD, and PyroMark Q96 ID. Not for use with PyroMark Q24 Advanced.

‡ For use with PyroMark Q24, PyroMark Q24 Advanced, PyroMark Q96 MD, and PyroMark Q96 ID.

§ For use with PyroMark Q24 Vacuum Workstation and PyroMark Q96 Vacuum Workstation.

Product	Contents	Cat. no.
PyroMark Q24 Plate (100)	24-well sequencing reaction plate	979201
PyroMark Q24 Control Oligo	For installation check of system	979203
PyroMark Q24 Validation Oligo	For performance check of system	979204
PCR kits and reagents		
PyroMark PCR Kit (200)*	For 200 reactions: 2x PyroMark PCR Master Mix (includes HotStarTaq DNA Polymerase and optimized PyroMark Reaction Buffer containing 3 mM $MgCl_2$ and dNTPs), 10x CoralLoad® Concentrate, 5x Q-Solution®, 25 mM $MgCl_2$, and RNase-Free Water	978703
EpiTect® Fast DNA Bisulfite Kit (50)*	For 50 preps: Bisulfite Solution, DNA Protect Buffer, MinElute® DNA Spin Columns, Carrier RNA, Buffers	59824
EpiTect Fast 96 Bisulfite Kit*	2 x EpiTect 96-well Plates, Bisulfite Solution, DNA Protect Buffer, Carrier RNA, Buffers	59720
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

* Other kit sizes/formats, available; see www.qiagen.com.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Trademarks: QIAGEN®, CoralLoad®, EpiTect®, MinElute®, PyroMark®, Pyrosequencing®, Pyrogram®, Q-Solution® (QIAGEN Group); ABgene® (Thermo Fisher Scientific); Eppendorf® (Eppendorf AG); Milli-Q® (Merck KGaA); Sepharose® (GE Healthcare); Variomag® (Thermo Electron LED GmbH).

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the PyroMark Q24 Advanced CpG Reagents to the following terms:

1. The PyroMark Q24 Advanced CpG Reagents may be used solely in accordance with the *PyroMark Q24 Advanced CpG Reagents Handbook* and for use with components contained in the Reagents only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of these Reagents with any components not included within these Reagents except as described in the *PyroMark Q24 Advanced CpG Reagents Handbook* and additional protocols available at www.qiagen.com.

2. Other than expressly stated licenses, QIAGEN makes no warranty that these Reagents and/or their use(s) do not infringe the rights of third-parties.

3. These Reagents and their components are licensed for one-time use and may not be reused, refurbished, or resold.

4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the Reagents agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Reagents and/or their components.

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