March 2015

PyroMark® Gold Q24 Reagents Handbook

Σ 5 x 24

Version 1

IVD

For performing Pyrosequencing® reactions on the PyroMark Q24 MDx

For in vitro diagnostic use

CE

REF 971802

HB 1057419EN

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Contents

Kit Contents

PyroMark Gold Q24 Reagents	**(5 x 24)**
Catalog no.	**971802**
Number of preps	**5 x 24**
Enzyme Mixture	1 vial
Substrate Mixture	1 vial
dATPαS	1180 µl
dGTP	1180 µl
dCTP	1180 µl
dTTP	1180 µl
Handbook	1

Symbols

 <N>	Contains reagents for <N> tests
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number

Global Trade Item Number

Temperature limitations

Legal manufacturer

Refer to information given in the handbook

Important note

Storage

Store nucleotides and freeze-dried enzyme and substrate (light sensitive) mixtures at 4–8°C. The nucleotides and freeze-dried reagents are stable at 4–8°C until the expiration date. The reconstituted enzyme and substrate mixtures are stable for at least 5 days at 4–8°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°C to –15°C. Frozen reagents should not be subjected to more than 3 freeze–thaw cycles.

Nucleotides must never be frozen.

Intended Use

PyroMark Q24 Gold Reagents are intended to be used with the PyroMark Q24 MDx system in in vitro diagnostic Pyrosequencing applications.

Product Use Limitations

For in vitro diagnostic use, the PyroMark Q24 MDx system may only be operated by

- personnel who have received special education and training with regard to procedures utilizing in vitro diagnostic medical devices, and
- accredited medical testing laboratories.

All operations must be performed according to PyroMark Q24 MDx system instructions, as provided through dialog messages appearing on the screen of the PyroMark Q24 MDx, the associated user manuals, handbooks, and technical support from QIAGEN, and within the limits set by the technical specifications.

Materials for sample preparation before Pyrosequencing analysis are not included in the product.

The product is intended solely for use on the PyroMark Q24 MDx system.

Strict compliance with the instrument user manual and this handbook 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.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

Results from the PyroMark Q24 MDx system must be interpreted within the context of all relevant clinical and laboratory findings.

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 Gold Q24 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).

Quality Control

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

Warnings and precautions

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

CAUTION: 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 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).

The following hazard and precautionary statements apply to components of PyroMark Gold Q24 Reagents.

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.

Introduction

PyroMark Gold Q24 Reagents are a set of reagents that is optimized for Pyrosequencing technology. The reagents are designed to generate a Pyrogram® with sharp and distinct peaks and low background. PyroMark Gold Q24 Reagents especially improve assays with longer sequencing read lengths, such as with CpG methylation analysis, as well as providing optimal conditions for mutation and SNP analyses.

PyroMark Gold Q24 Reagents contain all enzymes, substrates, and nucleotides that are needed in the Pyrosequencing cascade. Proper use of the reagents results in release of pyrophosphate and subsequent generation of a detectable light signal proportional to the number of nucleotides incorporated.

PyroMark Gold Q24 Reagents are intended to be used together with the PyroMark Q24 MDx system and the PyroMark Q24 Cartridge.

Enzyme mix

The enzyme mixture contains all enzymes that are needed in the Pyrosequencing cascade. These include DNA polymerase for incorporation of nucleotides, ATP sulfurylase for conversion of pyrophosphate to ATP, luciferase for generation of the light signal, and apyrase 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 secondary structures in the template.

Substrate mixture

The substrate mixture consists of adenosine 5' phosphosulfate (APS) needed for generation of ATP and luciferin, which serves as a substrate for luciferase in the light-generating part of the Pyrosequencing cascade.

Nucleotides

Nucleotides included in the PyroMark Gold Q24 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 deoxyadensine triphosphate (dATP) since it is efficiently used by the DNA polymerase but not recognized by the luciferase.

ⓘ Nucleotides must never be frozen.

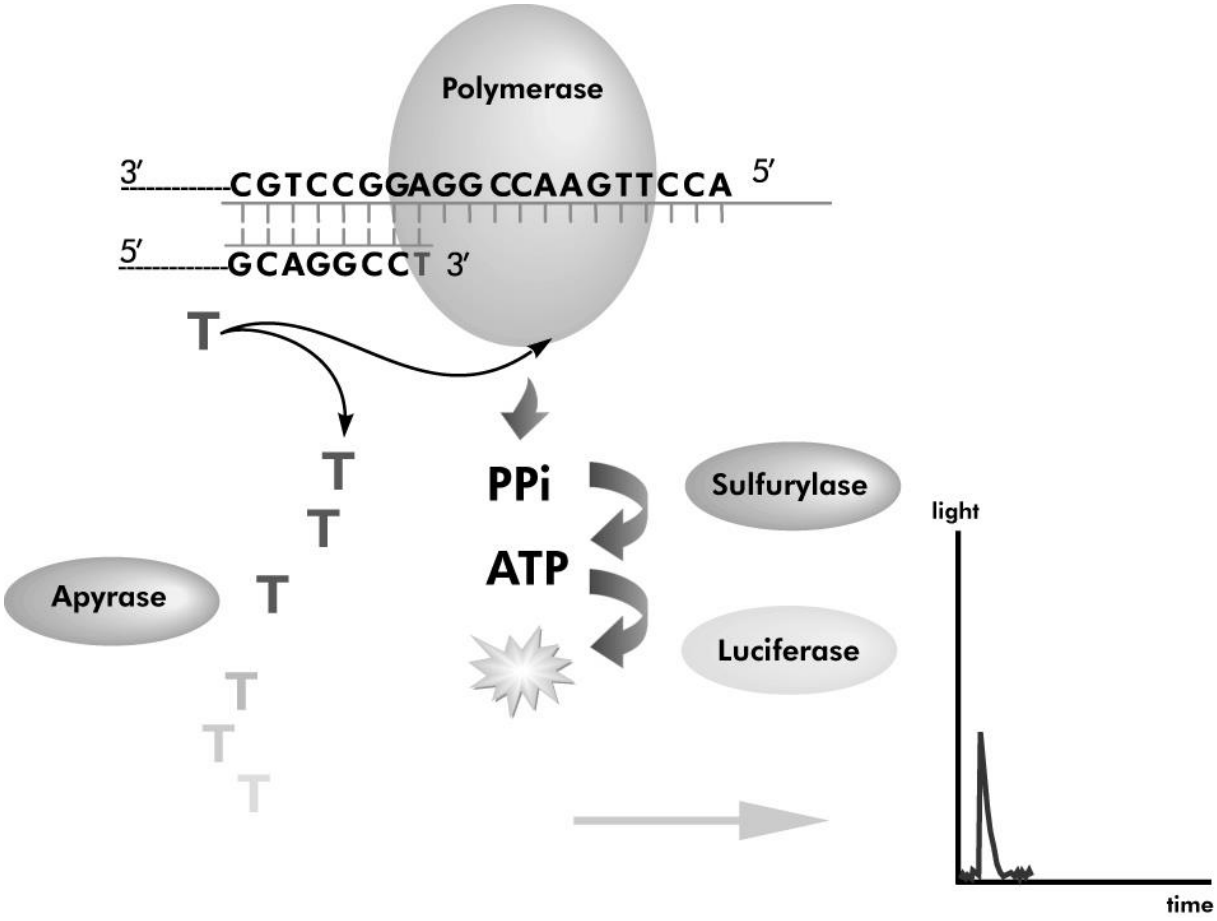

Figure 1. Schematic illustration of the Pyrosequencing cascade.

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.

- Pipets (adjustable)*
- Sterile pipet tips with filters
- PyroMark Q24 MDx (cat. no. 9001513)*†
- PyroMark Q24 MDx Software (cat. no. 9019063)†
- PyroMark Q24 MDx Vacuum Workstation (cat. no. 9001515 or 9001517)*†
- PyroMark Q24 Cartridge (cat. no. 979302)†
- PyroMark Q24 Control Oligo (cat. no. 979303)† for installation check of PyroMark Q24 MDx system
- PyroMark Q24 Validation Oligo (cat. no. 979304)† for performance check of PyroMark Q24 MDx system
- High-purity water (Milli-Q® 18.2 MΩ x cm or equivalent)
- Lint-free tissue

* 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.

Protocol: Loading PyroMark Q24 Cartridge with PyroMark Gold Q24 Reagents

This protocol describes loading PyroMark Gold Q24 Reagents into the PyroMark Q24 Cartridge before performing Pyrosequencing analysis on the PyroMark Q24 MDx.

Important point before starting

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

Procedure

1. **Open the PyroMark Gold Q24 Reagents box and remove the vials containing freeze-dried enzyme and substrate mixtures, and the vials containing nucleotides.**
2. **Dissolve the freeze-dried enzyme and substrate mixtures in 620 µl each of high-purity water (Milli-Q 18.2 MΩ x cm or equivalent, filtered through a 0.22 µm filter).**
3. **Mix by swirling the vial gently.**

   Do not vortex!

   In order to ensure that the mixture is fully dissolved, leave it at room temperature (15–25°C) for 5–10 min. 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.
4. **Allow the reagents and the PyroMark Q24 Cartridge to reach ambient temperature (20–25°C).**
5. **Place the PyroMark Q24 Cartridge with the label facing you.**
6. **Pipet the reagents into the PyroMark Q24 Cartridge according to Figure 2.**

   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.

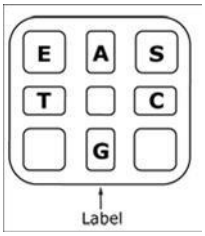

Figure 2. Illustration of the PyroMark Q24 Cartridge seen from above. The annotations correspond to the label on the reagent vials. Add enzyme mixture (**E**), substrate mixture (**S**), and nucleotides (**A**, **T**, **C**, **G**) according to the volume information given in the Pre Run information report, found in the "Tools" menu at run setup.

7. **Switch on the PyroMark Q24 MDx instrument.**
   The power switch is located at the rear of the instrument.
8. **Open the cartridge gate and insert the filled PyroMark Q24 Cartridge with the label facing out. Push the cartridge in fully and then push it down.**
9. **Ensure that the line is visible in front of the cartridge and close the gate.**
10. **Open the plate-holding frame, and place the plate on the heating block.**
11. **Close the plate-holding frame and the instrument lid.**
12. **Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.**

    ⓘ Do not remove the USB port before the run is finished.
13. **Select "Run" in the main menu (using the ▲ and ▼ screen buttons) and press "OK".**
14. **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".
15. **When the run file is selected, press "Select" to start the run.**
16. **When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press "Close".**
17. **Remove the USB memory stick.**
18. **Open the instrument lid.**
19. **Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.**
20. **Close the gate.**
21. **Discard solutions remaining in the PyroMark Q24 Cartridge.**
22. **Rinse the PyroMark Q24 Cartridge 4 times with high-purity water.**
23. **Spray the outside of the needles with high-purity water.**

24. To rinse the needles, fill the compartments completely with high-purity water and hold the cartridge over a sink while pressing firmly on top of each compartment with a finger. Check that the needle is clear. A jet of water should squirt straight out of the tip of the needle.

25. If the needle is blocked, follow step 25a. If the jet of water comes out of the needle at an angle, instead of straight out of the needle, follow step 25b. If the needle is clear, proceed to step 26.

25a. If the needle is blocked (for example, if the reagent cartridge has been left overnight without cleaning), fill the compartments with high-purity water, and immerse the PyroMark Q24 Cartridge in a beaker of high-purity water that covers the needles. Leave the reagent cartridge in the beaker for 1 h, rinse it, and repeat step 24.

25b. If the jet of water comes out at an angle, refill the compartment with water and repeat. If the water still comes out at an angle, discard the PyroMark Q24 Cartridge.

26. When all needles have been rinsed and checked, discard the water and let the PyroMark Q24 Cartridge dry on a lint-free tissue.

27. When the PyroMark Q24 Cartridge is dry, store it in a dust-free place.

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 User Manual* for general troubleshooting of the instrument.

	Comments and suggestions
Small or missing peaks in the Pyrogram	
a) Reagents incorrectly diluted or stored	ⓘ Be sure to follow the instructions in "Storage", page 5, and "Protocol: Loading PyroMark Q24 Cartridge with PyroMark Gold Q24 Reagents", page 11.
b) 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). Follow the kit handbook supplied with the PyroMark Kit used.
c) Blocked or damaged nucleotide needles in the PyroMark Q24 Cartridge	ⓘ Clean the PyroMark Q24 Cartridge and check that it is working correctly.
d) No enzyme or substrate added to the well (noted as a missing Pyrosequencing signal and missing peaks in the program)	ⓘ Clean the PyroMark Q24 Cartridge and check that it is working correctly.

	Comments and suggestions
e) Obstructed or damaged reagent 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.
f) PyroMark Q24 Cartridge incorrectly inserted	ⓘ Ensure that the cartridge is inserted correctly.
Poor or faulty sequence	
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 or QIAGEN authorized distributors. ⓘ Use the zoom in function to check if any peaks have been generated (select a section of Pyrogram with the left mouse button).

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 Gold Q24 Reagents (5 x 24)	For 5 x 24 samples for use on the PyroMark Q24 MDx: Enzyme Mixture, Substrate Mixture, and Nucleotides	971802
Accessories		
PyroMark Q24 Cartridge (3)	Cartridges for dispensing nucleotides and reagents on the PyroMark Q24 MDx	979302
Related products		
PyroMark Q24 MDx	Sequence based detection platform for Pyrosequencing of 24 samples in parallel	9001513
PyroMark Q24 MDx Vacuum Workstation	Vacuum Workstation (220 V) for preparing 24 samples in parallel, from PCR product to single-stranded template	9001515* 9001517†
PyroMark Q24 MDx Software	Application software	9019063
PyroMark Annealing Buffer (250 ml)	For annealing sequencing primer to single-stranded PCR product and for Pyrosequencing reaction	979309
PyroMark Binding Buffer (200 ml)	For binding of biotinylated PCR product to Sepharose® beads	979306
PyroMark Wash Buffer, concentrate (200 ml)	For washing of single-stranded DNA	979308
PyroMark Denaturation Solution (500 ml)	For denaturation of double-stranded PCR product into single-stranded template DNA	979307
PyroMark Q24 Plate (100)	24-well sequencing reaction plate	979301
PyroMark Q24 Control Oligo	For installation check of system	979303

* For rest of world (not UK).

† For the UK.

Product	Contents	Cat. no.
PyroMark Q24 Validation Oligo	For performance check of system	979304

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.

Limited License Agreement

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

1. The PyroMark Gold Q24 Reagents may be used solely in accordance with the *PyroMark Gold Q24 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 Gold Q24 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.
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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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Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 021-3865-3865 ■ Fax 021-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 02-33430-420 ■ Fax 02-33430-426 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 1544 7145 ■ Fax 1544 7146 ■ Technical 1544 7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-639

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Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

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