

July 2014

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# PyroMark<sup>®</sup> Gold Q96 Reagents Handbook

For performing Pyrosequencing<sup>®</sup> reactions on  
the PyroMark Q96 ID, PyroMark Q96 MD,  
and PyroMark Q96 MD Automated



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Sample & Assay Technologies

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## Kit Contents

<b>PyroMark Gold Q96 Reagents</b>	<b>5 x 96 (96)</b>	<b>50 x 96 (96)</b>	<b>SQA (96)</b>	<b>CDT (96)</b>
<b>Catalog no.</b>	<b>972804</b>	<b>972807</b>	<b>972812</b>	<b>972824</b>
<b>Number of preps (PyroMark Q96 ID)</b>	<b>5 x 96</b>	<b>–</b>	<b>1 x 96</b>	<b>–</b>
<b>Number of preps (PyroMark Q96 MD)</b>	<b>15 x 96</b>	<b>50 x 96</b>	<b>–</b>	<b>6 x 96</b>
<b>Number of preps (PyroMark Q96 MD Automated)</b>	<b>–</b>	<b>50 x 96</b>	<b>–</b>	<b>–</b>
Enzyme Mixture	5 vials	5 or 14 vials*	1 vial	2 vials
Substrate Mixture	5 vials	5 or 14 vials*	1 vial	2 vials
dATP $\alpha$ S	1400 $\mu$ l	5 x 620 $\mu$ l	1400 $\mu$ l	850 $\mu$ l
dGTP	1400 $\mu$ l	5 x 620 $\mu$ l	1400 $\mu$ l	850 $\mu$ l
dCTP	1400 $\mu$ l	5 x 620 $\mu$ l	1400 $\mu$ l	850 $\mu$ l
dTTP	1400 $\mu$ l	5 x 620 $\mu$ l	1400 $\mu$ l	850 $\mu$ l
Handbook	1	1	1	1

\* The packaging for this reagent has been updated. PyroMark Gold Q96 Reagents 50 x 96 (cat. no. 972807) containing Enzyme Mixture with material number 1074244 and Substrate Mixture with material number 1074248 now contain 14 vials of these reagents.

## Storage

Store nucleotides, lyophilized enzyme mixture, and lyophilized substrate mixture (light sensitive) at 2–8°C. The nucleotides and lyophilized reagents are stable at this storage temperature until the expiration date. When reconstituted, the enzyme and substrate reagents 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 –20°C. Frozen reagents should not be subjected to more than 3 freeze–thaw cycles.

**IMPORTANT:** Do not freeze the nucleotides.

## Product Use Limitations

PyroMark Gold Q96 Reagents are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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](http://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 Gold Q96 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](http://www.qiagen.com/Support) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://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](http://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,<sup>†</sup> or COSHH<sup>‡</sup> 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).

<sup>†</sup> ACGIH: American Conference of Government Industrial Hygienists (United States of America).

<sup>‡</sup> COSHH: Control of Substances Hazardous to Health (United Kingdom).

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of PyroMark Gold Q96 Reagents is tested against predetermined specifications to ensure consistent product quality.

## Introduction

PyroMark Gold Q96 Reagents are a set of reagents optimized for Pyrosequencing technology. The reagents are designed to generate Pyrogram<sup>®</sup> traces with sharp and distinct peaks and low background. PyroMark Gold Q96 Reagents improve assays with longer sequencing read-length, such as CpG methylation analysis and species identification, and provide optimal conditions for mutation and single nucleotide polymorphism (SNP) analysis.

PyroMark Gold Q96 Reagents contain all essential compounds — enzymes, substrates and nucleotides — needed in the Pyrosequencing cascade (Figure 1). All reagents are formulated to generate a clear, visible light signal proportional to the number of nucleotides incorporated into the analyzed DNA strand.

### Enzyme mixture

The enzyme mixture contains all enzymes that are needed in the Pyrosequencing cascade: DNA polymerase for incorporation of nucleotides, ATP sulfurylase for conversion of pyrophosphate to ATP, luciferase for generation of the light signal, and apyrase for degradation of unincorporated nucleotides and ATP. Single-strand binding protein (SSB) has been added to the mixture to disrupt secondary structures in the template, thereby increasing sequencing read-length.

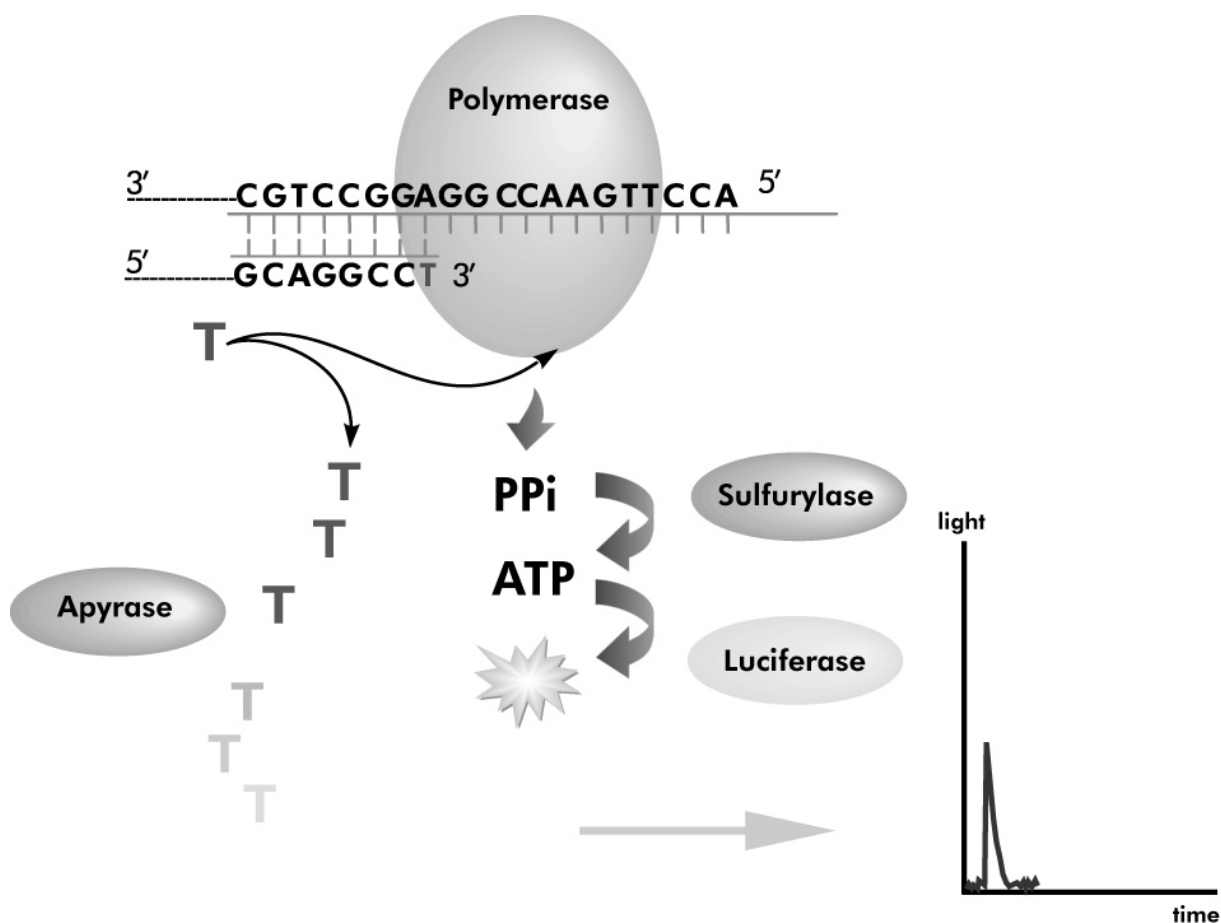
### Substrate mixture

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

### Nucleotides

Nucleotides included in PyroMark Gold Q96 Reagents are dissolved in a stabilizing buffer to prevent degradation. The concentration of nucleotides provided in each PyroMark Gold Q96 Reagents Kit is optimized for the kit's intended use. Thus, nucleotide solutions are more concentrated in the PyroMark Gold Q96 SQA Reagents and the PyroMark Gold Q96 Reagents (5 x 96) and (50 x 96) and more dilute in the PyroMark Gold Q96 CDT Reagents. It should be noted that deoxyadenosine alfa-thio triphosphate (dATP $\alpha$ S) is used as a substitute for deoxyadenosine triphosphate (dATP) since it is efficiently used by DNA polymerase, but not recognized by the luciferase.





**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.

## Description of protocols

Four versions of PyroMark Gold Q96 Reagents are available. The appropriate protocol to follow will depend on the instrument being used, the reagent dispensing unit of the instrument (reagent cartridge, dispensing tip holder, or capillary tip holder), and the number of preps (see “Kit Contents”, page 4).

- PyroMark Gold Q96 Reagents (5 x 96) can be used on the PyroMark Q96 ID for 5 x 96 preps, or on the PyroMark Q96 MD for 15 x 96 preps.
- PyroMark Gold Q96 Reagents (50 x 96) can be used on the PyroMark Q96 MD or on the PyroMark Q96 MD Automated for 50 x 96 preps.
- PyroMark Gold Q96 CDT Reagents are used on the PyroMark Q96 MD in conjunction with PyroMark Q96 HS Capillary Tips and the PyroMark Q96 HS Capillary Tip Holder for 6 x 96 preps.
- PyroMark Gold Q96 SQA Reagents are intended to be used on the PyroMark Q96 ID for 1 x 96 preps.

The protocol “Loading PyroMark Gold Q96 Reagents into the PyroMark Q96 Cartridge”, page 12, is for use with PyroMark Gold Q96 Reagents (5 x 96) or PyroMark Q96 SQA Reagents on the PyroMark Q96 ID.

The protocol “Loading PyroMark Gold Q96 Reagents into the PyroMark Q96 Dispensing Tip Holder”, page 14, is for use with PyroMark Gold Q96 Reagents (5 x 96) on the PyroMark Q96 MD, or with PyroMark Gold Q96 Reagents (50 x 96) on the PyroMark Q96 MD or the PyroMark Q96 MD Automated.

The protocol “Loading PyroMark Gold Q96 CDT Reagents into the PyroMark Q96 Capillary Tip Holder”, page 17, is for use with PyroMark Gold Q96 CDT Reagents on the PyroMark Q96 MD in conjunction with the PyroMark Q96 HS Capillary Tip Holder.

## 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.

### For use with the PyroMark Q96 ID

- PyroMark Q96 ID (see ordering information, page 25)
- PyroMark Q96 ID Software (see ordering information, page 25)
- PyroMark Q96 Cartridge (cat. no. 979004)

### For use with the PyroMark Q96 MD or PyroMark Q96 MD Automated and the PyroMark Q96 HS Dispensing Tip Holder

- PyroMark Q96 MD or MD Automated (see ordering information, page 25)
- PyroMark Q96 MD Software (see ordering information, page 25)
- PyroMark Q96 HS Dispensing Tip Holder (cat. no. 9019075)
- PyroMark Q96 HS Nucleotide Tip (cat. no. 979103)
- PyroMark Q96 HS Reagent Tip (cat. no. 979102)
- PyroMark Q96 HS Tip Storage Box (cat. no. 9019074)

### For use with the PyroMark Q96 MD and the PyroMark Q96 HS Capillary Tip Holder

- PyroMark Q96 MD (see ordering information, page 25)
- PyroMark Q96 MD Software (see ordering information, page 25)
- PyroMark Q96 HS Capillary Tip Holder (cat. no. 9019076)
- PyroMark Q96 HS Capillary Tip (cat. no. 979104)
- PyroMark Q96 HS Reagent Tip (cat. no. 979102)
- PyroMark Q96 HS Tip Storage Box (cat. no. 9019074)

### For all protocols

- PyroMark Q96 Vacuum Workstation (see ordering information, page 25)
- High purity water (Milli-Q® 18.2 MΩ x cm or equivalent)
- Pipets (adjustable)
- Pipet tips

# Protocol: Loading PyroMark Gold Q96 Reagents into the PyroMark Q96 Cartridge

This protocol describes the process for loading PyroMark Gold Q96 Reagents (5 x 96) or PyroMark Gold Q96 SQA Reagents (for 1 x 96 preps) into the PyroMark Q96 Cartridge. These instructions should be followed before performing Pyrosequencing analysis on the PyroMark Q96 ID. Brief cleaning procedures are also provided. For more detailed instructions, see the *PyroMark Q96 Cartridge Product Sheet*.

## Important points before starting

- PyroMark Q96 ID Software provides the volume of nucleotides, enzyme mixture, and substrate mixture needed for a specific run. In the Browser area of the PyroMark Q96 ID Software click “View” and choose “Run” to see these volumes (for more information, see *PyroMark Q96 ID Software Online Help*).
- **IMPORTANT:** Do not freeze the nucleotides.

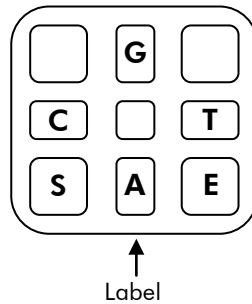
## Procedure

- 1. Dissolve lyophilized enzyme and substrate mixtures in 620  $\mu$ l each of high-purity water (Milli-Q 18.2 M $\Omega$  x cm or equivalent, filtered through a 0.22  $\mu$ M filter).**
- 2. Mix by swirling the vials gently. Do not vortex.**

To ensure that the mixtures are fully dissolved, leave at room temperature (15–25°C) for 5–10 min. Make sure that the solution is not turbid before filling the PyroMark Q96 Cartridge. If the reagents are not to be used immediately, place the vials on ice\* or in a refrigerator.
- 3. Allow the mixtures and reagent cartridge to reach ambient temperature (20–25°C).**
- 4. Position the reagent cartridge with the label facing you.**
- 5. Pipet the reagents into the cartridge compartments according to Figure 2, page 13.**

**Note:** Make sure that no air bubbles are transferred from the pipet to the cartridge.
- 6. Open the instrument lid and the dispensing unit cover of the PyroMark 96 ID, and insert the filled cartridge with the label facing out (See the *PyroMark Q96 ID User Manual*).**

\* 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. Schematic diagram of the PyroMark Q96 Cartridge (viewed from above).**

The annotations correspond to the label on the reagent vials. Add enzyme mixture, substrate mixture, and nucleotides according to the volumes given in the PyroMark Q96 ID Software (see the *PyroMark Q96 ID Software Online Help*).

7. Ensure that the cartridge is properly inserted (a line should be visible on the front of the cartridge) and close the dispensing unit cover.
8. Follow the instructions in the *PyroMark Q96 ID User Manual* to prepare and start a Pyrosequencing run.
9. When the run is finished, open the instrument and dispensing unit cover, and remove the reagent cartridge.
10. Discard the solutions remaining in the PyroMark Q96 Cartridge.
11. Rinse the cartridge 4 times with high-purity water.
12. Spray the outside of the cartridge needles with high-purity water.
13. To rinse the needles, fill the compartments completely with high-purity water and hold the cartridge over a sink while pressing firmly with a finger on top of each compartment (wear gloves). Check that each needle is clear. A jet of water should come straight out of the tip of the needle.
 

**Note:** If the needle is blocked, fill the compartments with high-purity water and place the cartridge in a beaker filled with enough water to cover the needles. Leave the cartridge in the beaker for 1 h, rinse it, and repeat steps 12 and 13.
14. Check that the jet of water coming out of each needle is straight (parallel to the direction of the needle). If the jet of water comes out at an angle, refill the compartment with water and apply pressure to generate a new jet of water. If the water still comes out at an angle, discard the PyroMark Q96 Cartridge.
15. When all needles have been rinsed and checked, discard the water and allow the cartridge to dry on a lint-free tissue.
16. When the PyroMark Q96 Cartridge is dry, store it in a dust-free place.

# Protocol: Loading PyroMark Gold Q96 Reagents into the PyroMark Q96 Dispensing Tip Holder

This protocol describes the process for loading PyroMark Gold Q96 Reagents (5 x 96) or PyroMark Gold Q96 Reagents (50 x 96) into reagent tips and nucleotide tips in the PyroMark Q96 Dispensing Tip Holder. These instructions should be followed before performing Pyrosequencing analysis on the PyroMark Q96 MD or the PyroMark Q96 MD Automated. Note that PyroMark Gold Q96 Reagents (5 x 96) includes sufficient reagents for 15 x 96 preps when used on the PyroMark Q96 MD. Brief cleaning procedures are also provided. For more detailed instructions, see the *PyroMark Q96 HS Reagent Tip Product Sheet* and the *PyroMark Q96 HS Nucleotide Product Sheet*.

## Important points before starting

- The packaging for PyroMark Gold Q96 Reagents (50 x 96) has been updated. The Enzyme Mixture and Substrate Mixture vials now have reduced content compared to the previous kit. Therefore, the reconstitution volume has also been reduced. Please check the material numbers on the labels of your Enzyme Mixture and Substrate Mixture vials, and use the volume indicated for your specific kit.
- PyroMark Q96 MD Software provides the volume of nucleotides, enzyme mixture, and substrate mixture needed for a specific run. In the Browser area of the PyroMark Q96 MD Software, click “View” and choose “Run” to see these volumes (for more information, see the *PyroMark Q96 MD Software Online Help*).
- Make sure to mark reagent tips and nucleotide tips with annotations corresponding to the label on the reagent vials.
- During the cleaning procedure, avoid overfilling the nucleotide tips and bringing the nucleotide tip heads in contact with water. Water may damage their hydrophobic coating.
- **IMPORTANT:** Do not freeze the nucleotides.

## Procedure

- 1. Dissolve the lyophilized Enzyme and Substrate Mixtures in the volume of high-purity water specified in the following table (Milli-Q 18.2 MΩ x cm or equivalent, filtered through a 0.22 μM filter). Use the volume that corresponds to your particular material numbers of Enzyme and Substrate Mixtures.**

	PyroMark Enzyme Mixture		PyroMark Substrate Mixture	
<b>Material number*</b>	<b>1056477</b>	<b>1074244</b> or <b>1056478</b>	<b>1056460</b>	<b>1074248</b> or <b>1056459</b>
Reconstitution volume	1550 $\mu$ l	620 $\mu$ l	1550 $\mu$ l	620 $\mu$ l

\* The material numbers are located on the enzyme and substrate mixture tube labels.

**2. Mix by swirling the vials gently. Do not vortex.**

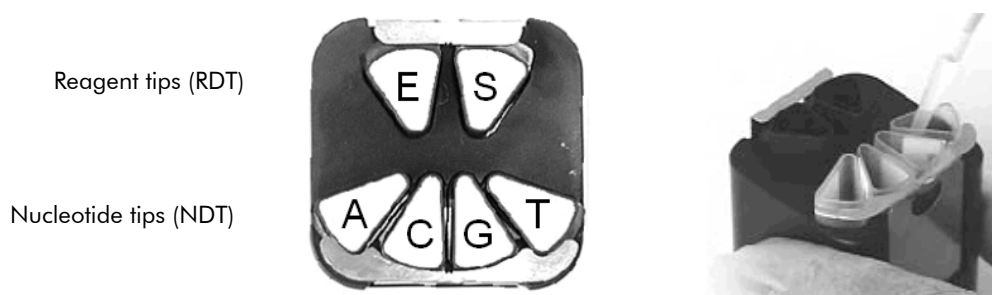
To ensure that the mixtures are fully dissolved, leave at room temperature (15–25°C) for 5–10 min. Make sure that the solution is not turbid before filling the dispensing tips. If the reagents are not to be used immediately, place the vials on ice<sup>†</sup> or in a refrigerator.

**3. Allow the mixtures and dispensing tip holder to reach ambient temperature (20–25°C).**

**4. Pull the springs apart on the dispensing tip holder and insert 2 PyroMark Q96 HS Reagent Tips (RDT) and 4 PyroMark Q96 HS Nucleotide Tips (NDT) into the slots according to Figure 3.**

**5. Pipet reagents into the appropriate dispensing tips according to Figure 3.**

**Note:** Make sure that no air bubbles are transferred from the pipet to the reagent tips (RDT) or nucleotide tips (NDT). Pipet the reagents into the narrow part of the dispensing tip.



**Figure 3. Arrangement of tips in the PyroMark Q96 Dispensing Tip Holder.** The annotations correspond to the label on the reagent vials. Add enzyme mixture, substrate mixture, and nucleotides according to the volumes given in the PyroMark Q96 MD Software (see the *PyroMark Q96 MD Software Online Help*).

<sup>†</sup> 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.

6. Open the instrument lid and the dispensing unit cover of the PyroMark Q96 MD or PyroMark Q96 MD Automated, and insert the filled dispensing tip holder with the label facing out (see the *PyroMark Q96 MD or PyroMark Q96 MD Automated User Manual*).
7. Follow the instructions in the *PyroMark Q96 MD User Manual* to prepare and start a Pyrosequencing run.
8. When the run is finished, remove the dispensing tip holder from the dispensing unit of the instrument.
9. Remove the reagent tips (RDT) from the dispensing tip holder by gently pulling the holder springs apart, and place the reagent tips in an empty dispensing tip holder.
10. Discard the solutions remaining in the reagent tips (RDT).
11. Rinse the reagent tips 3 times with high-purity water.
12. To rinse the tip heads, completely fill the tips with high-purity water and hold the tip holder over a sink while pressing firmly with a finger on top of the large opening of each tip (wear gloves). A spray or droplet of water should form at the end of each tip head.  
**Note:** If a tip head is blocked, place the tip head into a drop of water on a finger and repeat step 12. For more detailed instructions, see the *PyroMark Q96 HS Reagent Tip Product Sheet*.
13. Discard any water remaining in the reagent tips (RDT) and remove the tips from the dispensing tip holder.
14. Allow the dispensing tip holder and reagent tips (RDT) to dry.  
**Note:** Make sure that the outside of the tip heads is dry before reusing.
15. Discard the solutions remaining from the nucleotide tips (NDT).
16. Carefully rinse the nucleotide tips (NDT) twice with high-purity water.  
**IMPORTANT:** Make sure that the nucleotide tip heads do not come in contact with water. If a tip head comes in contact with water, press the tip head gently against a lint-free tissue placed at the bottom of the Q96 HS Tip Storage Box. For more detailed instructions, see the *PyroMark Q96 HS Nucleotide Tip Product Sheet*.
17. Remove the nucleotide tips (NDT) from the dispensing tip holder.
18. Allow the nucleotide tips (NDT) and dispensing tip holder to dry. To avoid contamination, nucleotide tips should dry on lint-free tissue in a PyroMark Q96 HS Tip Storage Box for at least 3 h before the next run.



# Protocol: Loading PyroMark Gold Q96 CDT Reagents into the PyroMark Q96 Capillary Tip Holder

This protocol describes the process for loading PyroMark Gold Q96 CDT Reagents into reagent tips and capillary tips in the PyroMark Q96 Capillary Tip Holder (for 6 x 96 preps). These instructions should be followed before performing Pyrosequencing analysis on the PyroMark Q96 MD. Brief cleaning procedures are also provided. For more detailed instructions, see the *PyroMark Q96 HS Reagent Tip Product Sheet* and the *PyroMark Q96 HS Capillary Tip Product Sheet*.

## Important points before starting

- PyroMark Q96 MD Software provides the volume of nucleotides, enzyme mixture, and substrate mixture needed for a specific run. In the Browser area of the PyroMark Q96 MD Software, click “View” and choose “Run” to see these volumes (for more information, see the *PyroMark Q96 MD Software Online Help*).
- Make sure to mark reagent tips and capillary tips with annotations corresponding to the label on the reagent vials.
- **IMPORTANT:** Do not freeze the nucleotides.

## Procedure

- 1. Dissolve lyophilized enzyme and substrate mixtures in 620  $\mu$ l each of high-purity water (Milli-Q 18.2 M $\Omega$  x cm or equivalent, filtered through a 0.22  $\mu$ M filter).**
- 2. Mix by swirling the vials gently. Do not vortex.**  
To ensure that the mixtures are fully dissolved, leave at room temperature (15–25°C) for 5–10 min. Make sure that the solution is not turbid before filling the dispensing tips. If the reagents are not to be used immediately, place the vials on ice\* or in a refrigerator.
- 3. Allow the mixtures and capillary tip holder to reach ambient temperature (20–25°C).**
- 4. Pull the springs apart on the capillary tip holder and insert 2 PyroMark Q96 HS Reagent Tips (RDT) and 4 PyroMark Q96 HS Capillary Tips (CDT) into the slots according to Figure 4, page 18.**

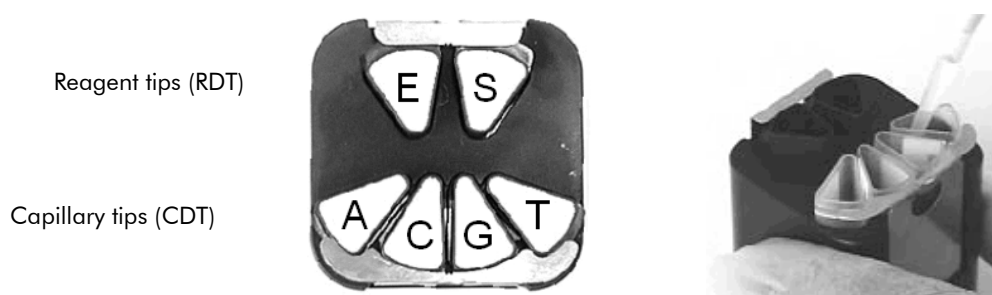
\* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- 5. Pipet enzyme mixture and substrate mixture into the reagent tips (RDT) according to Figure 4.**

**Note:** Make sure that no air bubbles are transferred from the pipet to the reagent tips. Pipet the reagents into the narrow part of the reagent tip.

- 6. Pipet double the volume of nucleotides given in the PyroMark Q96 MD Software into the capillary tips (CDT) according to Figure 4.**

**Note:** Make sure that no air bubbles are transferred from the pipet to the capillary tips. Pipet the reagents into the narrow part of the reagent tip.



**Figure 4. Arrangement of tips in the PyroMark Q96 Capillary Tip Holder.** The annotations correspond to the label on the reagent vials. Add enzyme mixture, substrate mixture, and nucleotides according to the volumes given in the PyroMark Q96 MD Software (see the *PyroMark Q96 MD Software Online Help*).

- 7. Open the instrument lid and the dispensing unit cover of the PyroMark Q96 MD, and insert the filled capillary tip holder with the label facing out (See the *PyroMark Q96 MD User Manual*).**
- 8. Follow the instructions in the *PyroMark Q96 MD User Manual* to prepare and start a Pyrosequencing run.**
- 9. When the run is finished, remove the capillary tip holder from the dispensing unit of the instrument.**
- 10. Discard the solutions remaining in the reagent tips (RDT) and capillary tips (CDT).**
- 11. Rinse the reagent tips (RDT) and capillary tips (CDT) 3 times with high-purity water.**

**12. To rinse the tip heads of the reagent tips (RDT) and the needles of the capillary tips (CDT), completely fill the tips with high-purity water and hold the tip holder over a sink while pressing firmly with a finger on top of the large opening of each tip (wear gloves). A spray or droplet of water should form at the end of each tip head and needle.**

**Note:** If the head of a reagent tip or needle of a capillary tip is blocked, place the tip head or needle into a drop of water on a finger and repeat step 12. For more detailed instructions, see the *PyroMark Q96 HS Reagent Tip Product Sheet* and the *PyroMark Q96 HS Capillary Tip Product Sheet*.

**13. Discard any water remaining in the reagent (RDT) and capillary (CDT) tips.**

**14. Remove the tips from the capillary tip holder by pulling apart the springs and lifting out the tips.**

**15. Allow the reagent tips (RDT), capillary tips (CDT), and capillary tip holder to dry.**

**Note:** Make sure that the outside of the tip heads and needles is dry before reusing.

## 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](http://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](http://www.qiagen.com)).

### Comments and suggestions

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#### Low or missing peaks in the Pyrogram trace after analysis with the PyroMark Q96 ID

- |  |  |
|--|--|
| a) Reagents incorrectly diluted or stored                          | Be sure to follow the instructions supplied with PyroMark Q96 Gold Reagents.   |
| b) One or several of the nucleotides was/were pipetted incorrectly | Be sure to add enough reagents. To determine the reagent volumes needed, click "View" and choose "Run" in the Browser area of the PyroMark Q96 ID Software.  |
| c) No enzyme mixture or substrate mixture was added to the well    | Clean the reagent cartridge and check that the needles that dispense enzyme and substrate mixture are not blocked or damaged (see the <i>PyroMark Q96 Cartridge Product Sheet</i> ).   |
| d) Obstructed or damaged cartridge needles                         | Clean the reagent cartridge and check all needles are not blocked or damaged.<br><br>In case of bent or damaged needles, discard the cartridge according to national, state, and local health and safety regulations for disposal of laboratory waste. |
| e) Reagent cartridge is inserted incorrectly                       | Ensure that the cartridge is inserted correctly into the dispensing unit.  |

#### Poor or faulty sequence

- |  |  |
|--|--|
| Contaminated sample has led to unusually high consumption of reagents (noted as a high presequencing signal) | Change buffers and do not use buffers other than those supplied by QIAGEN.<br><br>Use the "Zoom" function to check if any peaks have been generated (select an area of the Pyrogram trace with the left mouse button). |
|--|--|

## Comments and suggestions

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### Low or missing peaks in the Pyrogram trace after analysis with the PyroMark Q96 MD or PyroMark Q96 MD Automated

- |   |   |
|---|---|
| a) Reagents incorrectly diluted or stored   | Be sure to follow the instructions supplied with PyroMark Q96 Gold Reagents.  |
| b) One or several of the nucleotides were pipetted incorrectly                    | Be sure to add enough reagents. To determine the reagent volumes needed, click "View" and choose "Run" in the Browser area of the PyroMark Q96 MD Software.   |
| c) One of the nucleotide tips (NDT) or capillary tips (CDT) is blocked or damaged | Clean the nucleotide tip or capillary tip and ensure it is working correctly (see the <i>PyroMark Q96 HS Nucleotide Tip Product Sheet</i> or the <i>PyroMark Q96 HS Capillary Tip Product Sheet</i> ).<br><br>In case of damaged tips, discard according to federal, state, and local environmental regulations for disposal of laboratory waste. |
| d) No enzyme mixture or substrate mixture was added to the well                   | Clean the reagent tips (RDT) and check that they are working properly (see the <i>PyroMark Q96 HS Reagent Tip Product Sheet</i> ).  |
| e) Obstructed or damaged reagent tip head (RDT)                                   | Clean the reagent tips and check that they are working correctly.<br><br>In case of damaged or bent tip heads, discard the tips according to national, state, and local health and safety regulations for disposal of laboratory waste.   |
| f) The dispensing tip holder or capillary tip holder is inserted incorrectly      | Ensure that the dispensing tip holder or capillary tip holder is inserted correctly into the instrument.  |

### Poor or faulty sequence

- |   |  |
|---|--|
| Contaminated sample has led to unusually high consumption of substrate (noted as a high presequencing signal) | Change buffers and do not use buffers other than those supplied by QIAGEN.<br><br>Use the "Zoom" function to check if any peaks have been generated (select an area of the Pyrogram trace with the left mouse button). |
|---|--|

## References

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## Ordering Information

Product	Contents	Cat. no.
PyroMark Gold Q96 Reagents (5 x 96)	Enzyme mixture, substrate mixture, and nucleotides for performing Pyrosequencing reactions on the PyroMark Q96 ID (5 x 96) and PyroMark Q96 MD (15 x 96)	972804
PyroMark Gold Q96 Reagents (50 x 96)	Enzyme mixture, substrate mixture, and nucleotides for performing Pyrosequencing reactions on the PyroMark Q96 MD or PyroMark Q96 MD Automated	972807
PyroMark Gold Q96 SQA Reagents (1 x 96)	Enzyme mixture, substrate mixture, and nucleotides for performing Pyrosequencing reactions on the PyroMark Q96 ID	972812
PyroMark Gold Q96 CDT Reagents (6 x 96)	Enzyme mixture, substrate mixture, and nucleotides for performing Pyrosequencing reactions on the PyroMark Q96 MD using capillary dispensing tips (CDT)	972824
<b>Accessories</b>		
PyroMark Q96 Cartridge (3)	For delivery of nucleotides and reagents; for use with PyroMark Q96 ID	979004
PyroMark Q96 HS Dispensing Tip Holder	Reusable holder for tips, nucleotide dispensing tips, and reagent dispensing tips; for use with PyroMark Q96 MD	9019075
PyroMark Q96 HS Capillary Tip Holder	Reusable holder for tips, capillary dispensing tips, and reagent dispensing tips; for use with PyroMark Q96 MD	9019076
PyroMark Q96 HS Reagent Tip (4)	Reusable tips (4 in each package); for dispensing reagents (RDT); for use with PyroMark Q96 MD	979102
PyroMark Q96 HS Nucleotide Tip (8)	Reusable tips (8 in each package); for dispensing nucleotides (NDT); for use with PyroMark Q96 MD	979103

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
PyroMark Q96 HS Capillary Tip (8)	Reusable capillary tips (8 in each package); for dispensing nucleotides (CDT); for use with PyroMark Q96 MD	979104
PyroMark Q96 HS Tip Storage Box	For dust-free and safe storage of tips in between runs; for use with PyroMark Q96 MD	9019074
<b>Related products</b>		
PyroMark Q96 ID	Instrument for genetic analysis using Pyrosequencing technology	9001525
PyroMark Q96 ID Software	Application software for SQA, AQ, and SNP analysis	9019083
PyroMark Q96 ID Software (5)	Application software for SQA, AQ, and SNP analysis. (5 licenses)	9019084
PyroMark Q96 Plate Low (100)	For sample analysis of DNA template on PyroMark Q96 ID	979002
PyroMark Q96 MD	Instrument for genetic analysis using Pyrosequencing technology	9001526
PyroMark Q96 MD Automated	Instrument for genetic analysis using Pyrosequencing technology; automated version	9001527
PyroMark Q96 MD Software	Application software for AQ and SNP analysis	9019085
PyroMark Q96 MD Software (5)	Application software for AQ and SNP analysis (5 licenses)	9019086
PyroMark Q96 HS Plate (100)	For analysis of DNA template on PyroMark Q96 MD	979101
PyroMark Q96 Vacuum Workstation	Vacuum workstation for preparing 96 samples in parallel from PCR product to single-stranded template	varies*
PyroMark Annealing Buffer (250 ml) CE	Solution providing optimal conditions for annealing of sequencing primer to DNA template	979009

\* 9001529 (220 V); 9001528 (110 V); 9001740 (UK).



<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
PyroMark Binding Buffer (200 ml) CE	Solution providing optimal conditions for immobilization of biotinylated DNA to streptavidin-coated Sepharose <sup>®</sup> beads	979006
PyroMark Wash Buffer (conc. 200 ml) CE	Solution for use with the PyroMark Q96 Vacuum Workstation to wash and neutralize immobilized DNA	979008
PyroMark Denaturation Solution (500 ml) CE	Solution for use with the PyroMark Q96 Vacuum Workstation for preparation of single-stranded DNA template	979007

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