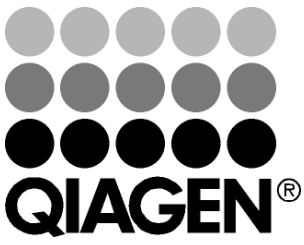
October 2009

PyroMark® Gold Q24 Reagents Handbook

For performing Pyrosequencing® reactions on the PyroMark Q24



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

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

PyroMark Gold Q24 Reagents	**(5 x 24)**
Catalog no.	**970802**
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

Storage

Store nucleotides, lyophilized enzyme mixture, and lyophilized substrate mixture (light sensitive) at 2–8°C. The nucleotides and lyophilized reagents are stable at 2–8°C until the expiration date. The 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 –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 Q24 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).

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.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/support/MSDS.aspx where you can find, view, and print the MSDS 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 Material Safety Data Sheets (MSDSs) 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 risk phrases apply to components of PyroMark Gold Q24 Reagents.

Substrate mixture

Contains dithiothreitol: Harmful if swallowed; irritating to eyes, respiratory system, and skin. Risk phrases:* R22-36/37/38

Contains luciferin: Toxic by inhalation, in contact with skin and if swallowed; causes eye, skin, and respiratory tract irritation. Risk phrases:* R23/24/25-36/37/38

Enzyme mixture

Contains tris(hydroxymethyl)aminomethane: Irritating to eyes, respiratory system and skin; danger of cumulative effects. Risk phrases:* R33-36/37/38

Contains dithiothreitol: Harmful if swallowed; irritating to eyes, respiratory system, and skin. Risk phrases:* R22-36/37/38

* R22: Harmful if swallowed; R23/24/25: Toxic by inhalation, in contact with skin and if swallowed; R33: Danger of cumulative effects; R36/37/38: Irritating to eyes, respiratory system and skin.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

PyroMark Gold Q24 Reagents are a set of reagents that is optimized for Pyrosequencing technology. The reagents are designed to generate Pyrogram® traces 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 (Figure 1). Proper use of the reagents results in release of pyrophosphate and 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 Instrument 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 and primers.

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. This ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin that generates visible light in amounts that are proportional to the amount of ATP.

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 luciferase.

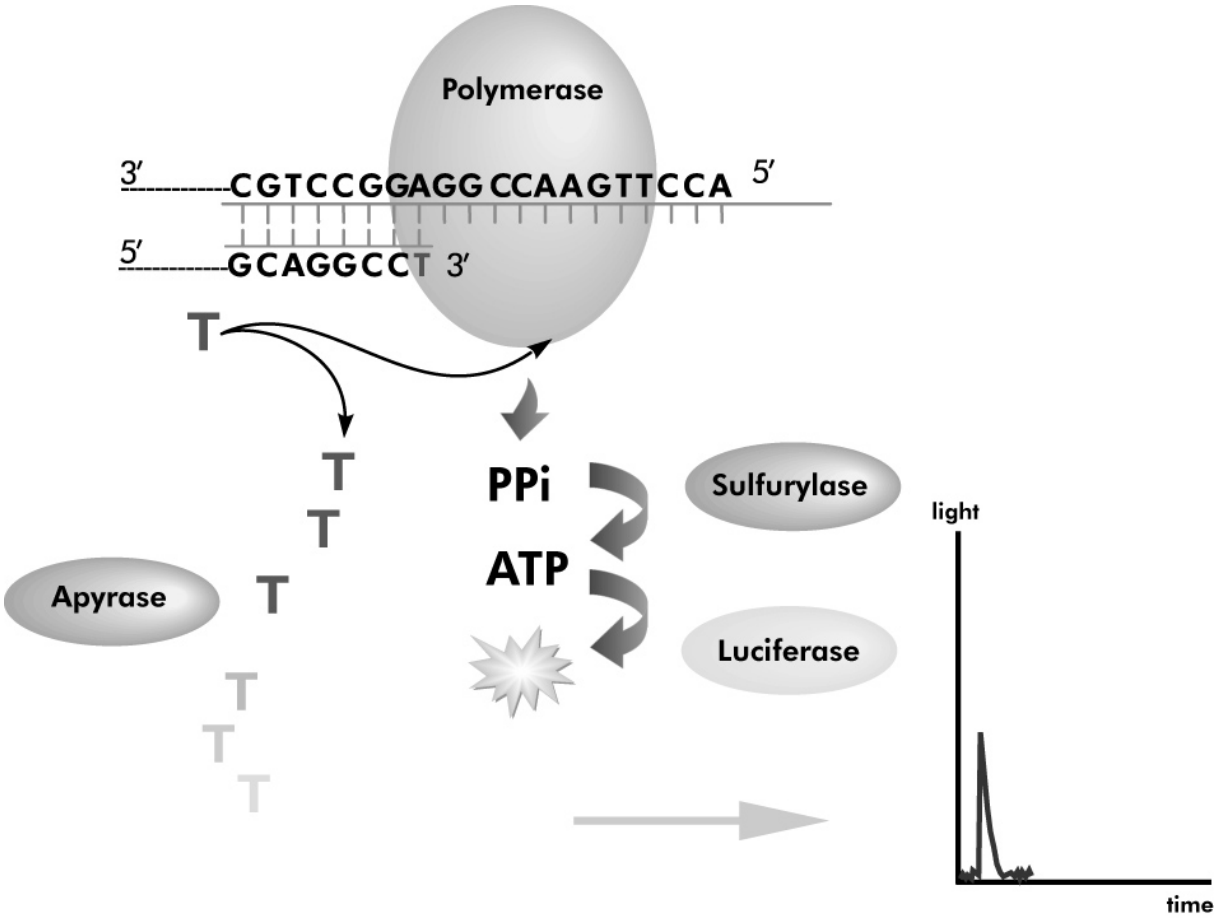


Figure 1. Schematic illustration of the Pyrosequencing cascade. As nucleotides are incorporated into the analyzed DNA strand, pyrophosphate is released and converted to ATP. Generated ATP drives the light reaction detected as a peak on the Pyrogram. Apyrase degrades any unincorporated nucleotides.

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
- PyroMark Q24 (cat. no. 9001514)*
- PyroMark Q24 Vacuum Workstation (cat. no. 9001518 [220V]; 9001516 [110V]; 9001519 [100V])*
- PyroMark Q24 Cartridge (cat. no. 979302)
- PyroMark Q24 Control Oligo (cat. no. 979203) for installation check of PyroMark Q24 system
- PyroMark Q24 Validation Oligo (cat. no. 979204) for performance check of PyroMark Q24 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.

Protocol: Loading PyroMark Gold Q24 Reagents into the PyroMark Q24 Cartridge

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

Important points 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 run.
- **IMPORTANT**: Do not freeze the nucleotides.

Procedure

1. **Open the PyroMark Gold Q24 Reagents box and remove the vials containing lyophilized enzyme and substrate mixtures, and the vials containing nucleotides.**
2. **Dissolve the lyophilized 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.

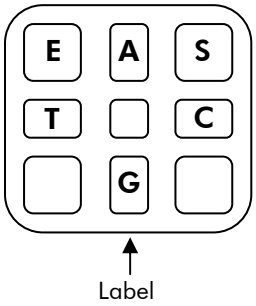


Figure 2. Illustration of the PyroMark Q24 Cartridge seen from above. The annotations correspond to the label on the reagent vials. Add enzyme mixture (**E**), substrate mixture (**S**), and nucleotides (**A**, **T**, **C**, **G**) according to the volume information given in the Pre Run information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

7. **Switch on the PyroMark Q24 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 you. Push the cartridge in fully and then push it down.**
9. **Ensure that the cartridge is properly inserted and close the gate.**
   Refer to the *PyroMark Q24 User Manual* for more information.
10. **Open the plate-holding frame, and place the PyroMark Q24 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 (wear gloves). Check that the needle is clear. A jet of water should come 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, (not parallel to the direction 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 enough 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 and the *PyroMark PCR Handbook* for PCR troubleshooting.

	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 4, and "Protocol: Loading PyroMark Gold Q24 Reagents into the PyroMark Q24 Cartridge", 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 of the PyroMark Q24 software). 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 presequencing signal and missing peaks in the Pyrogram)	Clean the PyroMark Q24 Cartridge and check that it is working correctly.
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.

	Comments and suggestions
f) PyroMark Q24 Cartridge incorrectly inserted	Ensure that the cartridge is inserted correctly.
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 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: Enzyme Mixture, Substrate Mixture, and Nucleotides	970802
Accessories		
PyroMark Q24 Cartridge (3)	Cartridges for dispensing nucleotides and reagents on the PyroMark Q24	979202
Related products		
PyroMark Q24	Sequence based detection platform for Pyrosequencing of 24 samples in parallel	9001514
PyroMark Q24 Vacuum Workstation	Workstation for preparing single-stranded DNA from 24 samples	Varies*
PyroMark Q24 Software	Application software	9019062
PyroMark Annealing Buffer (250 ml)†	For annealing sequencing primer to single-stranded PCR product and for Pyrosequencing reaction	979009
PyroMark Binding Buffer (200 ml)†	For binding of biotinylated PCR product to Streptavidin Sepharose® beads	979006
PyroMark Wash Buffer, concentrate (200 ml)‡	For washing of single-stranded DNA	979008
PyroMark Denaturation Solution (500 ml)	For denaturation of double-stranded PCR product into single-stranded template DNA	979007
PyroMark Q24 Plate (100)	24-well sequencing reaction plate	979201
PyroMark Q24 Control Oligo	For installation check of system	979203

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

† For use with PyroMark Q24, 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 Validation Oligo	For performance check of system	979204
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
PyroMark Assay Design SW 2.0†	Software for convenient design of PCR and sequencing primers, optimized for Pyrosequencing analysis	9019077

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

† Multiple software licenses available; please inquire.

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.
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.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ■ Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

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

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 65-67775366 ■ Fax 65-67785177 ■ Technical 65-67775366

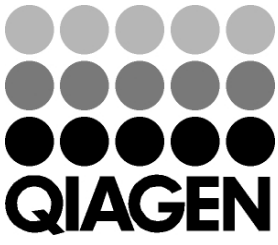
Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

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

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)



1060155 10/2009

Sample & Assay Technologies