
October 2015

PyroMark[®] Control Oligo Handbook

For use with PyroMark systems for installation
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Kit Contents

PyroMark Control Oligo	
Catalog no.	979203
Control Oligo 20 μ M	50 μ l
10x Dilution Buffer	2 x 1.7 ml
Handbook	1

Storage

The PyroMark Control Oligo should be stored at -15 to -30°C upon arrival. Repeated thawing and freezing (>5 x per year) should be avoided. The PyroMark Control Oligo is stable until the expiration date when stored under these conditions.

Intended Use

PyroMark Control Oligo is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of PyroMark Control Oligo 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 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.

Introduction

PyroMark Control Oligo provides a means to verify proper installation of PyroMark systems. In addition, PyroMark Control Oligo can be used in troubleshooting to determine if an unexpected result is related to the instrument, to the PyroMark Vacuum Workstations, or to the assay.

Principle and procedure

PyroMark Control Oligo is a biotinylated oligonucleotide, which allows the user to verify that all PyroMark instruments and PyroMark Vacuum Workstations are functioning properly.

Under defined conditions, the oligonucleotide can form an internal stem-loop structure. This structure enables self-priming of the oligonucleotide for extension by the DNA polymerase and eliminates the need for a sequencing primer in the Pyrosequencing® reaction. The sequenced region includes single bases of all nucleotides, homopolymers of 2 and 3 bases, and a wobbled/degenerated base. This variable position is automatically analyzed by the software, and results are presented as %C and %T. Figure 1 shows the structure of the oligonucleotide.

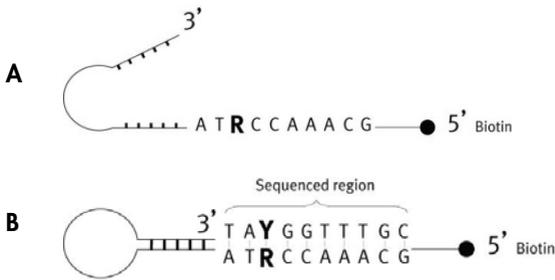


Figure 1. Structure of the PyroMark Control Oligo. [A] The open structure of the oligonucleotide. [B] The self-primed structure of the oligonucleotide, with the analyzed sequence indicated.

Description of protocols

It is recommended that 2 runs be performed to verify proper installation of PyroMark instruments.

Function of PyroMark Q24 or PyroMark Q24 Advanced

To verify correct function of PyroMark Q24 or PyroMark Q24 Advanced, follow “Protocol: Verifying the Function of PyroMark Q24 or PyroMark Q24 Advanced”, page 11. PyroMark Control Oligo is added directly to PyroMark Q24 Plate **without** prior preparation on the PyroMark Q24 Vacuum Workstation.

Function of PyroMark Q24 or PyroMark Q24 Advanced together with the PyroMark Q24 Vacuum Workstation

To verify correct function of PyroMark Q24 or PyroMark Q24 Advanced and PyroMark Q24 Vacuum Workstation, follow “Protocol: Verifying the Function of PyroMark Q24 or PyroMark Q24 Advanced and PyroMark Q24 Vacuum Workstation”, page 17. PyroMark Control Oligo is prepared using the PyroMark Q24 Vacuum Workstation before analysis on PyroMark Q24 or PyroMark Q24 Advanced.

Troubleshooting PyroMark Q24 or PyroMark Q24 Advanced together with the PyroMark Q24 Vacuum Workstation

To perform a troubleshooting of PyroMark Q24 or PyroMark Q24 Advanced and PyroMark Q24 Vacuum Workstation, follow “Protocol: Troubleshooting Procedure for PyroMark Q24 or PyroMark Q24 Advanced and PyroMark Q24 Vacuum Workstation”, page 26. A Pyrosequencing reaction is performed with 8 wells containing PyroMark Control Oligo and 8 wells containing PyroMark Control Oligo prepared on the PyroMark Q24 Vacuum Workstation.

Troubleshooting PyroMark Q96 ID and PyroMark Vacuum Workstation

To perform a troubleshooting of PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation, follow “Protocol: Troubleshooting Procedure for PyroMark Q96 ID and PyroMark Q96 Vacuum Workstation”, page 36. A Pyrosequencing reaction is performed with 8 wells containing PyroMark Control Oligo and 8 wells containing PyroMark Control Oligo prepared on the PyroMark Q96 Vacuum Workstation.

Troubleshooting PyroMark Q96 MD together with the PyroMark Q96 Vacuum Workstation

To perform a troubleshooting of PyroMark Q96 MD and PyroMark Q96 Vacuum Workstation, follow “Protocol: Troubleshooting Procedure for Q96 MD and PyroMark Q96 Vacuum Workstation”, page 45. A Pyrosequencing reaction is performed with 8 wells containing PyroMark Control Oligo and 8 wells containing PyroMark Control Oligo prepared on the PyroMark Q96 Vacuum Workstation.

Function of the PyroMark Q48 Autoprep

To verify correct function of PyroMark Q48 Autoprep, follow “Protocol: Verifying the Function of PyroMark Q48 Autoprep”, page 55.

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 PyroMark Q24 Systems

- PyroMark Q24 (cat. no. 9001514)*
- PyroMark Q24 Software (cat. no. 9019062)
- PyroMark Q24 Plate (100) (cat. no. 979201)
- PyroMark Q24 Cartridge (3) (cat. no. 979202)
- PyroMark Q24 Vacuum Workstation (cat. no. 9001518 [220V]; 9001516 [110V]; 9001519 [100V])*
- PyroMark Gold Q24 Reagents (5 x 24) (cat. no. 970802)

For use with PyroMark Q24 Advanced Systems

- PyroMark Q24 Advanced (cat. no. 9002270)*
- PyroMark Q24 Advanced Software (cat. no. 9022779)
- PyroMark Q24 Plate (100) (cat. no. 979201)
- PyroMark Q24 Cartridge (3) (cat. no. 979202)
- PyroMark Q24 Vacuum Workstation (cat. no. 9001518 [220V]; 9001516 [110V]; 9001519 [100V])*
- PyroMark Q24 Advanced Reagents (4 x 24) (cat. no. 970902)

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

For use with PyroMark Q96 ID Systems

- PyroMark Q96 ID (cat. no. 9001525)*
- PyroMark Q96 ID Software (cat. no. 9019083)
- PyroMark Q96 Plate Low (100) (cat. no. 979002)
- PyroMark Q96 Cartridge (3) (cat. no. 979004)
- PyroMark Gold Q96 Reagents (5x96) (cat. no. 972804)
- PyroMark Q96 Vacuum Workstation (cat. no. 9001529 [220 V]; 9001528 [110V]; 9001740 [UK])*

For use with PyroMark Q96 MD Systems

- PyroMark Q96 MD (cat. no. 9001526) *
- PyroMark Q96 MD Software (cat. no. 9019085)
- PyroMark Q96 HS Plate (100) (cat. no. 979101)
- PyroMark Q96 HS Dispensing Tip Holder (cat. no. 9019075)
- PyroMark Q96 HS Reagent Tips (4) (cat. no. 979102)
- PyroMark Q96 HS Nucleotide Tips (8) (cat. no. 979103)
- PyroMark Gold Q96 Reagents (5 x 96) (cat. no. 972804)
- PyroMark Q96 Vacuum Workstation (cat. no. 9001529 [220 V]; 9001528 [110V]; 9001740 [UK])*

For use with PyroMark Q48 Autoprep

- PyroMark Q48 Autoprep instrument (cat. no. 9002470, including PyroMark Q48 Cartridges and PyroMark Q48 Autoprep software)*

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

- PyroMark Q48 Discs (cat. no. 974901)
- PyroMark Q48 Advanced Reagents (cat. no. 974002) or PyroMark Q48 Advanced CpG Reagents (cat. no. 974022)
- PyroMark Q48 Magnetic Beads (cat. no. 974203)
- PyroMark Q48 Absorber Strips (cat. no. 974912)

Additional equipment needed for Q24 and Q96 systems

- PyroMark Binding Buffer (cat. no. 979006)
- PyroMark Denaturation Solution (cat. no. 979007)
- PyroMark Wash Buffer, concentrate (cat. no. 979008)
- PyroMark Annealing Buffer (cat. no. 979009)
- Plate mixer* for immobilization to beads
- Heating block* capable of attaining 80°C
- 24- or 96-well PCR plate or strips
- Strip caps
- Streptavidin Sepharose® High Performance (GE Healthcare, cat. no. 17-5113-01)

Additional equipment needed for all systems

- 1.5 ml or 2 ml microcentrifuge tubes for dilution of the PyroMark Control Oligo
- Pipets (adjustable)*
- Sterile pipet tips
- High-purity water (Milli-Q® 18.2 MΩ x cm or equivalent)
- Ethanol (70%)

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

Protocol: Verifying the Function of PyroMark Q24 or PyroMark Q24 Advanced

This protocol describes how to use the PyroMark Control Oligo to verify the function of PyroMark Q24 or PyroMark Q24 Advanced. To also verify the function of the PyroMark Q24 Vacuum Workstation, see “Protocol: Verifying the Function of PyroMark Q24 or PyroMark Q24 Advanced together with the PyroMark Q24 Vacuum Workstation”, page 17.

Important points before starting

- The procedure for PyroMark Q24 Systems differs slightly from the procedure for PyroMark Q24 Advanced Systems. If using a PyroMark Q24 System, follow the instructions marked with a triangle (▲). If using a PyroMark Q24 Advanced System, follow the instructions marked with a square (■).
- PyroMark Q24 Software cannot be used with PyroMark Q24 Advanced Reagents. PyroMark Q24 Advanced Software cannot be used with PyroMark Gold Q24 Reagents. Using the incorrect reagent and software combination will result in incorrect reagent dispensations and lead to inaccurate results.
- For further information on how to create an Assay Setup and a Run Setup, see the ▲ *PyroMark Q24 Software User Guide* or ■ *PyroMark Q24 Advanced Software User Guide*.

Things to do before starting

- Follow the instructions in ▲ *PyroMark Q24 User Manual* or ■ *PyroMark Q24 Advanced User Manual* to install the PyroMark Q24.
- The dilution buffer provided with the PyroMark Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Prewarm the PyroMark Q24 Plate Holder on a heating block at 80°C for use in step 14.

- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. Set up an assay for the PyroMark Control Oligo in the ▲ PyroMark Q24 Software or ■ PyroMark Q24 Advanced Software.
2. Click  in the toolbar and select **New AQ Assay**.
3. Type the following sequence in **Sequence to Analyze**: TAYGGTTTGC.
4. Click the **Generate Dispensation Order** icon to get the following nucleotide dispensation order: CTGACTGTG.

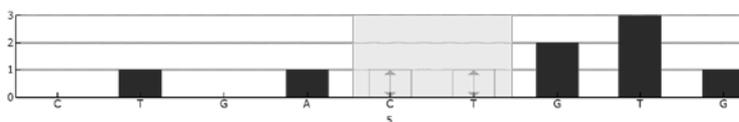


Figure 2. Histogram for AQ mode. Nucleotide additions 1 and 3 are blank dispensations and serve as negative controls. The fifth and the sixth dispensations analyze the variable position (wobbled/degenerated base).

5. Click  in the toolbar to save the assay.
6. Create a **Run Setup** by importing the assay parameters to all 24 wells.
To add an assay to a well, you can either:
 - Right-click the well and select **Load Assay** from the context menu, or
 - Select the assay in the shortcut browser, and click and drag the assay to the well.
 A well is color-coded according to the assay type loaded to the well.
7. Save the **Run Setup** to a USB memory stick (supplied with PyroMark Q24).
8. Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select **Pre Run Information** from the **Tools** menu and, when the report appears, click .

-
9. Load the PyroMark Q24 Cartridge with the appropriate volumes of ▲ PyroMark Gold Q24 Reagents or ■ PyroMark Q24 Advanced Reagents, as given in the **Pre Run Information** report from step 8.

Note: Make sure that no air bubbles are transferred from the pipet to the tips, and that all liquid is collected at the bottom of the cartridge. (See “Troubleshooting Guide”, page 60, for more information.)

The **Pre Run Information** report, found in the **Tools** menu at run setup (see the ▲ *PyroMark Q24 Software User Guide* or ■ *PyroMark Q24 Advanced Software User Guide*), provides information about the volume of nucleotides, enzyme mixture, and substrate mixture needed for the assay.

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.

10. 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.
11. Ensure that the cartridge is properly inserted and close the gate.
12. Dilute the PyroMark Control Oligo to 0.04 µM as shown in Table 1.

Table 1. Dilution of the PyroMark Control Oligo

Component	Volume	Concentration
PyroMark Control Oligo	10 μl	20 μM
1x Dilution buffer*	90 μl	–
First serial dilution	100 μl	2 μM
First serial dilution (from above)	30 μl	2 μM
▲ 1x Dilution buffer* or ■ PyroMark Q24 Advanced Annealing Buffer	1470 μl	–
Final dilution	1500 μl[†]	0.04 μM

* Make sure that the 10x dilution buffer supplied with the PyroMark Control Oligo is diluted with high-purity water before use. See “Things to do before starting”, page 17.

† Provides a sufficient amount for 60 PyroMark Q24 test reactions or 75 PyroMark Q24 Advanced test reactions.

13. Add ▲ 25 μl or ■ 20 μl of the diluted (0.04 μM) PyroMark Control Oligo to each well of a PyroMark Q24 Plate.
 14. Heat the PyroMark Q24 Plate with the PyroMark Oligo at 80°C for ▲ 2 min or ■ 5 min or using a heating block and the prewarmed PyroMark Q24 Plate Holder.
 15. ▲ Remove the PyroMark Q24 Plate from the plate holder, and let the samples cool to room temperature (15–25°C) for at least 5 min. Open the plate-holding frame and place the plate on the heating block of the PyroMark Q24.
 - Remove the PyroMark Q24 Plate together with the hot plate holder from the heating block. Open the plate-holding frame and place the plate immediately on the heating block of the PyroMark Q24. Proceed with the next steps to start the run immediately.
- Important:** If using a PyroMark Q24 Advanced System, the time between removing the plate holder from the heating block and placing the PyroMark Q24 plate in the PyroMark Q24 and starting the run should not exceed 30 seconds.
16. Close the plate-holding frame and the instrument lid.
 17. Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.

Do not remove the USB memory stick before the run is finished.
 18. Select **Run** in the main menu (using the ▲ and ▼ screen buttons) and press **OK**.

19. 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**.

20. When the run file is selected, press **Select** to start the run.

21. When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press **Close**.

22. Remove the USB memory stick.

23. Open the instrument lid.

24. Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.

25. Close the gate.

26. Open the plate-holding frame and remove the PyroMark Q24 Plate from the heating block.

27. Close the plate-holding frame and the instrument lid.

28. Discard the PyroMark Q24 Plate and clean the PyroMark Q24 Cartridge (see the ▲ *PyroMark Gold Q24 Reagents Handbook* or the ■ *PyroMark Q24 Advanced Reagents Handbook*).

29. Open the run in the ▲ PyroMark Q24 Software or the ■ PyroMark Q24 Advanced Software and analyze all wells. The peak pattern for Run 1 should look like the one in Figure 3.

To obtain peak height values, select **Export Peak Heights** from the **Tools** menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft® Excel® (Delimited), and calculate the mean single peak height for each well as described on the next page.

- **Perform a quality assessment.**

All wells should give "Passed" quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with %C indicated in a blue rectangle in the Pyrogram®. If the quality assessment is "Check" or "Failed", look in **Well Information** for explanations.

- **Evaluate peak heights.**

The single peak height (dispensations 2, 4, and 9) should be 95 ± 55 RLU.

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see “Troubleshooting Guide”, page 60, for possible reasons for the failure and rerun Run 1. If the repeat of Run 1 fails, 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).

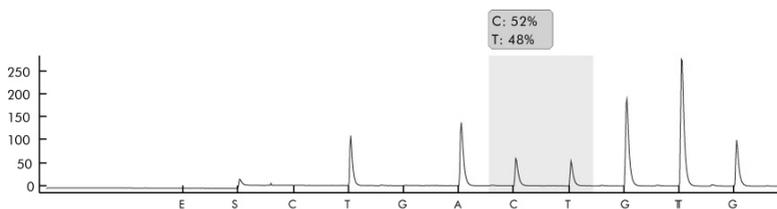


Figure 3. Pyrogram of Run 1.

Protocol: Verifying the Function of PyroMark Q24 or PyroMark Q24 Advanced together with the PyroMark Q24 Vacuum Workstation

This protocol describes how to use PyroMark Control Oligo to verify the function of PyroMark Q24 or PyroMark Q24 Advanced together with the PyroMark Q24 Vacuum Workstation. To verify the function of PyroMark Q24 or PyroMark Q24 Advanced only, see “Protocol: Verifying the Function of PyroMark Q24 or PyroMark Q24 Advanced”, page 11.

Important point before starting

- The procedure for PyroMark 24 Systems differs slightly from the procedure for PyroMark Q24 Advanced Systems. If using a PyroMark Q24 System, follow the instructions marked with a triangle (▲). If using a PyroMark Q24 Advanced System, follow the instructions marked with a square (■).
- PyroMark Q24 Software cannot be used with PyroMark Q24 Advanced Reagents. PyroMark Q24 Advanced Software cannot be used with PyroMark Gold Q24 Reagents. Using the incorrect reagent and software combination will result in incorrect reagent dispensations and lead to inaccurate results.
- For further information on how to create an **Assay Setup** and a **Run Setup**, see the ▲ *PyroMark Q24 Software User Guide* or ■ *PyroMark Q24 Advanced Software User Guide*.

Things to do before starting

- Follow the instructions in ▲ *PyroMark Q24 User Manual* or ■ *PyroMark Q24 Advanced User Manual* to install the PyroMark Q24.

- The dilution buffer provided with the PyroMark Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Prewarm the PyroMark Q24 Plate Holder on a heating block at 80°C for use in step 33.
- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. Set up an assay for the PyroMark Control Oligo by using the PyroMark Q24 Software.
2. Click  in the toolbar and select **New AQ Assay**.
3. Type the following sequence in **Sequence to Analyze**: TAYGGTTGCA.
4. Manually enter the following **Dispensation Order**: CGTTATCGTTGC.

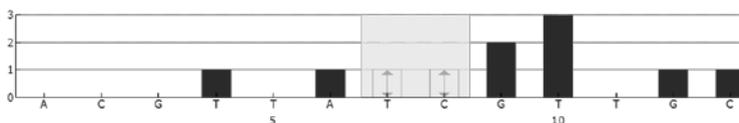


Figure 4. Histogram for AQ mode. Nucleotide additions 1, 2, 3, 5, and 11 are blank and serve as negative controls. The seventh and the eight dispensations analyze the variable position.

5. Click  in the toolbar to save the assay.
6. Create a **Run Setup** by importing the assay parameters to all 24 wells.
To add an assay to a well, you can either:
 - Right-click the well and select **Load Assay** from the context menu, or
 - Select the assay in the shortcut browser, and click and drag the assay to the well.
A well is color-coded according to the assay type loaded to the well.
7. Save the **Run Setup** to a USB memory stick (supplied with PyroMark Q24).
8. Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select **Pre Run Information** from the **Tools** menu and, when the report appears, click .

9. Gently shake the bottle containing Streptavidin Sepharose High Performance (HP) until it is a homogeneous solution.
10. Prepare a master mix for DNA immobilization. Check the lot number of the Streptavidin Sepharose HP, and use the volume of beads specified for the particular lot number. For ▲ PyroMark Q24 Systems, prepare the master mix according to Table 2. For ■ PyroMark Q24 Advanced Systems, use Table 3. Prepare a volume at least 10% greater than that required for the total number of reactions to be performed.

Table 2. ▲ Master mix for DNA immobilization using PyroMark Q24 Systems

Number of samples	For Sepharose lot number 10057037 or higher		For Sepharose lot numbers lower than 10057037	
	1	26*	1	26*
Streptavidin Sepharose High Performance	1 µl	26 µl	2 µl	52 µl
PyroMark Binding Buffer	40 µl	1040 µl	40 µl	1040 µl
High-purity water	14 µl	364 µl	13 µl	338 µl
Total volume	55 µl	1430 µl	55 µl	1430 µl

* Provides a sufficient amount for the 24 samples required.

Table 3. ■ Master mix for DNA immobilization using PyroMark Q24 Advanced Systems

Number of samples	For Sepharose lot number 10057037 or higher		For Sepharose lot numbers lower than 10057037	
	1	26*	1	26*
Streptavidin Sepharose High Performance	1 µl	26 µl	2 µl	52 µl
PyroMark Binding Buffer	40 µl	1040 µl	40 µl	1040 µl
High-purity water	19 µl	494 µl	18 µl	468 µl
Total volume	60 µl	1560 µl	60 µl	1560 µl

* Provides a sufficient amount for the 24 samples required.

11. Dilute the PyroMark Control Oligo to 0.04 μM as shown in Table 4.

Table 4. Dilution of the PyroMark Control Oligo

Component	Volume	Concentration
PyroMark Control Oligo	10 μl	20 μM
1x Dilution buffer*	90 μl	–
First serial dilution	100 μl	2 μM
First serial dilution (from above)	30 μl	2 μM
▲ 1x Dilution buffer* or ■ PyroMark Q24 Advanced Annealing Buffer	1470 μl	–
Final dilution	1500 μl[†]	0.04 μM

* Make sure that the 10x dilution buffer supplied with the PyroMark Control Oligo is diluted with high-purity water before use. See “Things to do before starting”, page 17.

[†] Provides a sufficient amount for 60 PyroMark Q24 test reactions or 75 PyroMark Q24 Advanced test reactions.

12. Shake the tube containing the master mix, and add ▲ 55 μl or ■ 60 μl of the master mix and ▲ 25 μl or ■ 20 μl of the diluted (0.04 μM) PyroMark Control Oligo to all 24 wells of a 24-well PCR plate or strips.

13. Seal the PCR plate (or strips) using strip caps.

14. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm.

Sepharose beads sediment quickly. Capturing of beads must take place immediately following agitation.

During this step, prepare the PyroMark Q24 Vacuum Workstation for sample preparation (see Appendix A, page 71).

15. Load the PyroMark Q24 Cartridge with the appropriate volumes of ▲ PyroMark Gold Q24 Reagents or ■ PyroMark Q24 Advanced Reagents, as given in the **Pre Run Information** report from step 8.

Note: Make sure that no air bubbles are transferred from the pipet to the tips, and that all liquid is collected at the bottom of the cartridge. (See “Troubleshooting Guide”, page 60, for more information.)

The **Pre Run Information** report, found in the **Tools** menu at run setup (see the ▲ *PyroMark Q24 Software User Guide* or ■ *PyroMark Q24 Advanced Software User Guide*), provides information about the volume of nucleotides, enzyme mixture, and substrate mixture needed for the assay.

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. 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.

16. 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.

17. Ensure that the cartridge is properly inserted and close the gate.

18. Add ▲ 25 µl of PyroMark Annealing Buffer or ■ 20 µl of PyroMark Advanced Annealing Buffer to each well of a PyroMark Q24 Plate.

Keep one of the PyroMark Q24 Plate Holders (supplied with the PyroMark Q24 Vacuum Workstation) at room temperature (15–25°C), and use it as support when preparing and moving the plate.

19. Place the PCR plate (or strips) and the PyroMark Q24 Plate on the worktable of the prepared PyroMark Q24 Vacuum Workstation (see Figure 5).

Ensure that the plate is in the same orientation as when samples were loaded.



Figure 5. Placement of PCR plate (or strips) and PyroMark Q24 Plate on the PyroMark Q24 Vacuum Workstation. The marked positions contain 70% ethanol (1), PyroMark Denaturation Solution (2), PyroMark Wash Buffer (3), and high-purity water (4, 5). P: Parking position.

20. Apply vacuum to the vacuum tool by opening the vacuum switch.
21. Carefully lower the filter probes into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.

Sephacrose beads sediment quickly. If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.
22. Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.
23. Transfer the tool to the trough containing PyroMark Denaturation Solution (trough 2). Flush the filter probes for 5 s.
24. Transfer the tool to the trough containing PyroMark Wash Buffer (trough 3). Flush the filter probes for 10 s.
25. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see **Figure 6**).



Figure 6. Illustration of the vacuum tool raised to beyond 90° vertical.

26. While the tool is held over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).
27. Release the beads in the plate containing ▲ 25 µl or ■ 20 µl PyroMark Annealing Buffer by shaking the tool from side to side. Allow the filter probes to rest on the bottom of the wells.
28. Transfer the tool to the first trough containing high-purity water (trough 4) and agitate the tool for 10 s.
29. Wash the filter probes by lowering the probes into the second trough with high-purity water (trough 5) and applying vacuum. Flush the probes with 70 ml high-purity water.

30. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 6).
31. Close the vacuum switch on the tool (Off), and place the tool in the Parking (P) position.
32. 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, see Appendix C, page 75.
33. Heat the PyroMark Q24 Plate with the PyroMark Oligo at 80°C for ▲ 2 min or ■ 5 min or using a heating block and the prewarmed PyroMark Q24 Plate Holder.
34. ▲ Remove the PyroMark Q24 Plate from the plate holder, and let the samples cool to room temperature (15–25°C) for at least 5 min. Open the plate-holding frame and place the plate on the heating block of the PyroMark Q24.
 - Remove the PyroMark Q24 Plate together with the hot plate holder from the heating block. Open the plate-holding frame and place the plate immediately on the heating block of the PyroMark Q24. Proceed with the next steps to start the run immediately.

Important: If using a PyroMark Q24 Advanced System, the time between removing the plate holder from the heating block and placing the PyroMark Q24 plate in the PyroMark Q24 and starting the run should not exceed 30 seconds.
35. Close the plate-holding frame and the instrument lid.
36. Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.

Do not remove the USB memory stick before the run is finished.
37. Select **Run** in the main menu (using the ▲ and ▼ screen buttons) and press **OK**.
38. 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**.
39. When the run file is selected, press **Select** to start the run.

40. When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press **Close**.
41. Remove the USB memory stick.
42. Open the instrument lid.
43. Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.
44. Close the gate.
45. Open the plate-holding frame and remove the PyroMark Q24 Plate from the heating block.
46. Close the plate-holding frame and the instrument lid.
47. Discard the PyroMark Q24 Plate and clean the PyroMark Q24 Cartridge (see the ▲ *PyroMark Gold Q24 Reagents Handbook* or the ■ *PyroMark Q24 Advanced Reagents Handbook*).
48. Open the run in the ▲ *PyroMark Q24 Software* or the ■ *PyroMark Q24 Advanced Software* and analyze all wells. The peak pattern for Run 2 should look like the one in Figure 7.

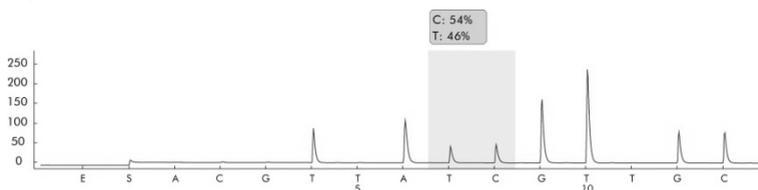


Figure 7. Pyrogram of Run 2.

49. Confirm the proper installation of the system and use of the reagents by evaluating the quality assessment, quantification results, single peak heights, and background.
To obtain peak height values, select **Export Peak Heights** from the **Tools** menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft Excel (Delimited), and calculate the mean single peak height and background for each well as described below.

- **Perform a quality assessment.**

All wells should give “Passed” quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with % C indicated in a blue rectangle in the Pyrogram. If the quality assessment is “Check” or “Failed”, look in **Well Information** for explanations.

- **Evaluate the quantification results.**

Select the **AQ Analysis Statistics Report** from the **Reports** menu. Quantification results are given in the report with standard deviation. The %C should be in the range 40–60%. The standard deviation should not exceed 2% units.

- **Evaluate single peak heights.**

The mean single peak height should be 95 ± 55 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 4, 6, 12, 13)}}{4}$$

- **Evaluate the background.**

Background from blank dispensations should not exceed 3%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 2, 3, 5)}}{\text{Sum single peaks (dispensation 4, 6, 12, 13)}} \times 100$$

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see “Troubleshooting Guide”, page 60, for possible causes and actions to be taken. If the troubleshooting guide does not explain the problem, 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).

Protocol: Troubleshooting Procedure for PyroMark Q24 or PyroMark Q24 Advanced together with the PyroMark Q24 Vacuum Workstation

If an unexpected result has been obtained it is crucial to determine whether this is related to PyroMark Q24 or PyroMark Q24 Advanced, the PyroMark Q24 Vacuum Workstation, or the assay. This protocol describes how to use PyroMark Control Oligo to verify the function of PyroMark Q24 or PyroMark Q24 Advanced, comparing results with or without the PyroMark Q24 Vacuum Workstation.

Important point before starting

- The procedure for PyroMark 24 Systems differs slightly from the procedure for PyroMark Q24 Advanced Systems. If using a PyroMark Q24 System, follow the instructions marked with a triangle (▲). If using a PyroMark Q24 Advanced System, follow the instructions marked with a square (■).
- PyroMark Q24 Software cannot be used with PyroMark Q24 Advanced Reagents. PyroMark Q24 Advanced Software cannot be used with PyroMark Gold Q24 Reagents. Using the incorrect reagent and software combination will result in incorrect reagent dispensations and lead to inaccurate results.
- For further information on how to create an **Assay Setup** and a **Run Setup**, see the ▲ *PyroMark Q24 Software User Guide* or ■ *PyroMark Q24 Advanced Software User Guide*.

Things to do before starting

- Follow the instructions in ▲ *PyroMark Q24 User Manual* or ■ *PyroMark Q24 Advanced User Manual* to install the PyroMark instrument.

- The dilution buffer provided with the PyroMark Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Prewarm the PyroMark Q24 Plate Holder on a heating block at 80°C for use in step 33.
- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. Set up an assay for the PyroMark Control Oligo by using the PyroMark Q24 Software.
2. Click  in the toolbar and select **New AQ Assay**.
3. Type the following sequence in **Sequence to Analyze**: TAYGGTTTGCA.
4. Manually enter the following **Dispensation Order**: ACGTTATCGTTGC.

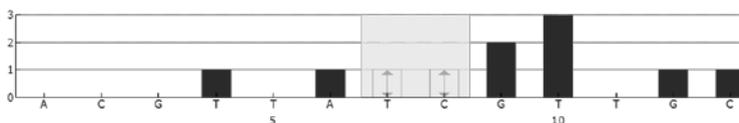


Figure 8. Histogram for AQ mode. Nucleotide additions 1, 2, 3, 5, and 11 are blank and serve as negative controls. The seventh and the eighth dispensations analyze the variable position.

5. Click  in the toolbar to save the assay.
6. Create a **Run Setup** by importing the assay parameters to the appropriate wells.

We recommend using 16 wells: 8 wells for samples prepared using the PyroMark Q24 Vacuum Workstation and 8 samples added directly to PyroMark Q24 Plate.

To add an assay to a well, you can either:

 - Right-click the well and select **Load Assay** from the context menu, or
 - Select the assay in the shortcut browser, and click and drag the assay to the well.

Recommended: Fill in Sample ID, Plate ID, Barcode, Reagent ID, and Run Note.

A well is color-coded according to the assay type loaded to the well.
7. Save the **Run Setup** to a USB memory stick (supplied with PyroMark Q24).

8. Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select **Pre Run Information** from the **Tools** menu and, when the report appears, click .
9. Gently shake the bottle containing Streptavidin Sepharose High Performance (HP) until it is a homogeneous solution.
10. Prepare a master mix for DNA immobilization. Check the lot number of the Streptavidin Sepharose HP, and use the volume of beads specified for the particular lot number. For ▲ PyroMark Q24 Systems, prepare the master mix according to Table 5. For ■ PyroMark Q24 Advanced Systems, use Table 6. Prepare a volume at least 10% greater than that required for the total number of reactions to be performed.

Table 5. ▲ Master mix for DNA immobilization using PyroMark Q24 Systems

Number of samples	For Sepharose lot number 10057037 or higher		For Sepharose lot numbers lower than 10057037	
	1	9*	1	9*
Streptavidin Sepharose High Performance	1 µl	9 µl	2 µl	18 µl
PyroMark Binding Buffer	40 µl	360 µl	40 µl	360 µl
High-purity water	14 µl	126 µl	13 µl	117 µl
Total volume	55 µl	495 µl	55 µl	495 µl

* Provides a sufficient amount for the 8 samples required.

Table 6. ■ Master mix for DNA immobilization using PyroMark Q24 Advanced Systems

Number of samples	For Sepharose lot number 10057037 or higher		For Sepharose lot numbers lower than 10057037	
	1	9*	1	9*
Streptavidin Sepharose High Performance	1 µl	9 µl	2 µl	18 µl
PyroMark Binding Buffer	40 µl	360 µl	40 µl	360 µl
High-purity water	19 µl	171 µl	18 µl	162 µl
Total volume	60 µl	540 µl	60 µl	540 µl

* Provides a sufficient amount for the 8 samples required.

11. Dilute the PyroMark Control Oligo to 0.04 μM as shown in Table 7.

Table 7. Dilution of the PyroMark Control Oligo

Component	Volume	Concentration
PyroMark Control Oligo	10 μl	20 μM
1x Dilution buffer*	90 μl	–
First serial dilution	100 μl	2 μM
First serial dilution (from above)	30 μl	2 μM
▲ 1x Dilution buffer* or ■ PyroMark Q24 Advanced Annealing Buffer	1470 μl	–
Final dilution	1500 μl	0.04 μM

* Make sure that the 10x dilution buffer supplied with the PyroMark Control Oligo is diluted with high-purity water before use. See “Things to do before starting”, page 26.

12. Shake the tube containing the master mix, and add ▲ 55 μl or ■ 60 μl of the master mix and ▲ 25 μl or ■ 20 μl of the diluted (0.04 μM) PyroMark Control Oligo to 8 wells of a 24-well PCR plate or strips.

13. Seal the PCR plate (or strips) using strip caps.

14. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm.

Sepharose beads sediment quickly. Capturing of beads must take place immediately following agitation.

During this step, prepare the PyroMark Q24 Vacuum Workstation for sample preparation (see Appendix A, page 71).

15. Load the PyroMark Q24 Cartridge with the appropriate volumes of ▲ PyroMark Gold Q24 Reagents or ■ PyroMark Q24 Advanced Reagents, as given in the **Pre Run Information** report from step 8.

Note: Make sure that no air bubbles are transferred from the pipet to the tips, and that all liquid is collected at the bottom of the cartridge. (See “Troubleshooting Guide”, page 60, for more information.)

The **Pre Run Information** report, found in the **Tools** menu at run setup (see the ▲ *PyroMark Q24 Software User Guide* or ■ *PyroMark Q24 Advanced Software User Guide*), provides information about the volume of nucleotides, enzyme mixture, and substrate mixture needed for the assay.

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. 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.

16. 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.

17. Ensure that the cartridge is properly inserted and close the gate.

18. Add ▲ 25 µl of PyroMark Annealing Buffer or ■ 20 µl of PyroMark Advanced Annealing Buffer to each well of the PyroMark Q24 Plate that will be used with the immobilized PyroMark Control Oligo to be processed with the PyroMark Q24 Vacuum Workstation. Add ▲ 25 µl or ■ 20 µl of the diluted (0.04 µM) PyroMark Q24 Control Oligo to 8 additional wells according to the Run Setup.

Keep one of the PyroMark Q24 Plate Holders (supplied with the PyroMark Q24 Vacuum Workstation) at room temperature (15–25°C), and use it as support when preparing and moving the plate.

19. Place the PCR plate (or strips) and the PyroMark Q24 Plate on the worktable of the PyroMark Q24 Vacuum Workstation (see Figure 9).

Ensure that the plate is in the same orientation as when samples were loaded.



Figure 9. Placement of PCR plate (or strips) and PyroMark Q24 Plate on the PyroMark Q24 Vacuum Workstation. The marked positions contain 70% ethanol (1), PyroMark Denaturation Solution (2), PyroMark Wash Buffer (3), and high-purity water (4, 5). P: Parking position.

20. Apply vacuum to the tool by opening the vacuum switch.
21. Carefully lower the filter probes into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.

Sepharose beads sediment quickly. If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.
22. Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.
23. Transfer the tool to the trough containing PyroMark Denaturation Solution (trough 2). Flush the filter probes for 5 s.
24. Transfer the tool to the trough containing PyroMark Wash Buffer (trough 3). Flush the filter probes for 10 s.
25. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 10).



Figure 10. Illustration of the vacuum tool raised to beyond 90° vertical.

26. While the tool is held over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).
27. Release the beads in the plate containing ▲ 25 µl or ■ 20 µl PyroMark Annealing Buffer by shaking the tool from side to side. Allow the filter probes to rest on the bottom of the wells.
28. Transfer the tool to the first trough containing high-purity water (trough 4) and agitate the tool for 10 s.
29. Wash the filter probes by lowering the probes into the second trough with high-purity water (trough 5) and applying vacuum. Flush the probes with 70 ml high-purity water.
30. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 10).
31. Close the vacuum switch on the tool (Off), and place the tool in the Parking (P) position.
32. 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, see Appendix C, page 75.
33. Heat the PyroMark Q24 Plate with the PyroMark Oligo at 80°C for ▲ 2 min or ■ 5 min or using a heating block and the prewarmed PyroMark Q24 Plate Holder.
34. ▲ Remove the PyroMark Q24 Plate from the plate holder, and let the samples cool to room temperature (15–25°C) for at least 5 min. Open the plate-holding frame and place the plate on the heating block of the PyroMark Q24.
 - Remove the PyroMark Q24 Plate together with the hot plate holder from the heating block. Open the plate-holding frame and place the plate immediately on the heating block of the PyroMark Q24. Proceed with the next steps to start the run immediately.

Important: If using a PyroMark Q24 Advanced System, the time between removing the plate holder from the heating block and placing the PyroMark Q24 plate in the PyroMark Q24 and starting the run should not exceed 30 seconds.
35. Close the plate-holding frame and the instrument lid.

-
36. Insert the USB memory stick (containing the run file) into the USB port at the front of the instrument.

Do not remove the USB memory stick before the run is finished.
 37. Select **Run** in the main menu (using the ▲ and ▼ screen buttons) and press **OK**.
 38. 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**.
 39. When the run file is selected, press **Select** to start the run.
 40. When the run is finished and the instrument confirms that the run file has been saved to the USB memory stick, press **Close**.
 41. Remove the USB memory stick.
 42. Open the instrument lid.
 43. Open the cartridge gate and remove the PyroMark Q24 Cartridge by lifting it up and pulling it out.
 44. Close the gate.
 45. Open the plate-holding frame and remove the PyroMark Q24 Plate from the heating block.
 46. Close the plate-holding frame and the instrument lid.
 47. Discard the PyroMark Q24 Plate and clean the PyroMark Q24 Cartridge (see the ▲ PyroMark Gold Q24 Reagents Handbook or the ■ PyroMark Q24 Advanced Reagents Handbook).
 48. Open the run in the ▲ PyroMark Q24 Software or the ■ PyroMark Q24 Advanced Software and analyze all wells. The peak pattern for Run 2 should look like the one in Figure 11.

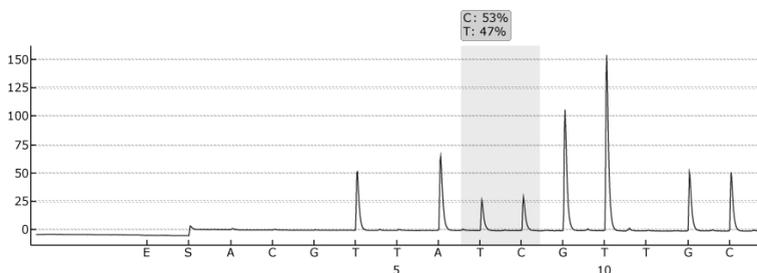


Figure 11. Pyrogram of PyroMark Q24 Troubleshooting Run.

49. Confirm the proper installation of the system and use of the reagents by evaluating the quality assessment, quantification results, single peak heights, and background.

To obtain peak height values, select **Export Peak Heights** from the **Tools** menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft Excel (Delimited), and calculate the mean single peak height and background for each well as described below.

- **Perform a quality assessment.**

All wells should give “Passed” quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with %C indicated in a blue rectangle in the Pyrogram. If the quality assessment is “Check” or “Failed”, look in **Well Information** for explanations.

- **Evaluate the quantification results.**

Select the **AQ Analysis Statistics Report** from the **Reports** menu. Quantification results are given in the report with standard deviation. The %C should be in the range 40–60%. The standard deviation should not exceed 2 percentage units.

- **Evaluate single peak heights.**

The mean single peak height should be 95 ± 55 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 4, 6, 12, 13)}}{4}$$

- **Evaluate the background.**

Background from blank dispensations should not exceed 3%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 2, 3, 5)}}{\text{Sum single peaks (dispensation 4, 6, 12, 13)}} \times 100$$

50. Evaluate the difference in peak heights with and without sample preparation. The reduction in peak height between samples prepared using the PyroMark Q24 Vacuum Workstation compared with PyroMark Control Oligo added directly to the PyroMark Q24 Plate should not be more than 20%.

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see "Troubleshooting Guide", page 60, for possible causes and actions to be taken. If the troubleshooting guide does not explain the problem, 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).

Protocol: Troubleshooting Procedure for PyroMark Q96 ID together with the PyroMark Q96 Vacuum Workstation

If an unexpected result has been obtained, it is crucial to determine whether this is related to PyroMark Q96 ID, the PyroMark Q96 Vacuum Workstation, or the assay. This protocol describes how to use PyroMark Control Oligo to verify the function of PyroMark Q96 ID and to compare results of samples prepared with or without the PyroMark Q96 Vacuum Workstation.

Important points before starting

- Assay and run files are set up using PyroMark Q96 ID Software 2.5.
- For further information on how to create an Assay Setup and a Run Setup, see the *PyroMark Q96 ID Software 2.5 Online Help*.
- **Note:** This protocol describes the procedure with the PyroMark Q96 ID Software 2.5. To use the PyroMark Q96 ID Software 1.0 or the PyroMark CpG Software, please see the corresponding Online Help sections for instructions on assay setup, run setup, and run evaluation.

Things to do before starting

- Follow the instructions in *PyroMark Q96 ID User Manual* to install the PyroMark Q96 ID.
- The dilution buffer provided with the PyroMark Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Place the PyroMark Q96 Sample Prep Thermoplate Low on a heating block at 80°C for use in step 31.

- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. Set up an assay for the PyroMark Control Oligo by using the PyroMark Q24 Software.
2. Click  in the toolbar and select **New AQ Assay**.
3. Type the following sequence in **Sequence to Analyze**: TAYGGTTTGCA.
4. Manually enter the following **Dispensation Order**: ACGTTATCGTTGC.

Note: For a thorough control of the instrument, sample preparation and reagents, additional blanks are included in the dispensing order.

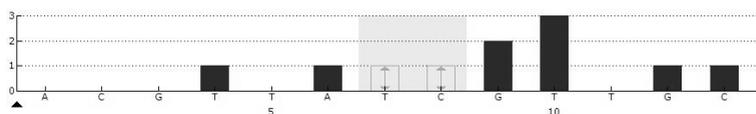


Figure 12. Histogram from PyroMark Q96 ID Software. Nucleotide additions 1, 2, 3, 5 and 11 are blank dispensations and serve as negative controls. The seventh and the eighth dispensations analyze the variable position (wobbled/degenerated base).

5. Click  in the toolbar to save the assay.
6. Create a **Run Setup** by importing the assay parameters to appropriate wells.

We recommend using 16 wells: 8 wells for samples prepared using the PyroMark Q96 Vacuum Workstation and 8 samples added directly to PyroMark Q96 Plate Low.

To add an assay to a well, you can either:

- Right-click the well and select **Load Assay** from the context menu, or
- Select the assay in the shortcut browser, then click and drag the assay to the well

Note: A well is color-coded according to the assay type loaded to the well.

7. Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup. Select **Pre Run Information** from the **Tools** menu and, when the report appears, click .

8. Gently shake the bottle containing Streptavidin Sepharose High Performance until it is a homogeneous solution.
9. Prepare a master mix for DNA immobilization according to Table 8. Check the lot number of the Streptavidin Sepharose HP. For lot number 10057037 or higher use 1.5 μl . For lot numbers lower than 10057037, use 3 μl . Prepare a volume 10% greater than that required for the total number of reactions to be performed.

Table 8. Master mix for DNA immobilization

Component	Volume per sample	
	For lot number 10057037 or higher	For lot numbers lower than 10057037
Streptavidin Sepharose HP	1.5 μl	3 μl
PyroMark Binding Buffer	38.5 μl	37 μl
Total volume	40 μl	40 μl

10. Dilute the PyroMark Control Oligo to 0.05 μM as shown in Table 9.

Table 9. Dilution of the PyroMark Control Oligo

Component	Volume	Concentration
PyroMark Control Oligo	10 μl	20 μM
1x Dilution buffer*	90 μl	–
First serial dilution	100 μl	2 μM
First serial dilution (from above)	20 μl	2 μM
1x Dilution buffer*	780 μl	–
Final dilution	800 μl	0.05 μM

* Make sure that the 10x dilution buffer supplied with the PyroMark Control Oligo is diluted with high-purity water before use. See “Things to do before starting”, page 36.

11. Add 40 μl of the master mix and 40 μl of the diluted (0.05 μM) PyroMark Control Oligo to 8 wells of a 96-well PCR plate or strips.
12. Seal the PCR plate (or strips) using strip caps.

13. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm.

During this step, prepare the PyroMark Q96 Vacuum Workstation for sample preparation (see Appendix B, page 73).

Note: Sepharose beads sediment quickly. Capturing of beads must take place immediately following agitation.

14. Add 40 µl of PyroMark Annealing Buffer to the wells of the PyroMark Q96 Plate Low that will be used with the immobilized PyroMark Control Oligo to be processed with the PyroMark Q96 Vacuum Workstation. Add 40 µl of the diluted (0.05 µM) PyroMark Control Oligo to 8 additional wells according to the Run Setup.

Since the oligonucleotide is self-primed, no sequencing primer is required. The beads are released into PyroMark Annealing Buffer.

15. Place the PCR plate (or strips) and the PyroMark Q96 Plate Low on the worktable of the PyroMark Q96 Vacuum Workstation (see Figure 13).

Ensure that the plate is in the same orientation as when samples were loaded.

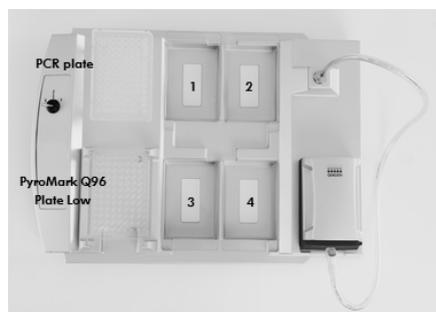


Figure 13. Placement of PCR plate (or strips) and PyroMark Q96 Plate Low on the PyroMark Q96 Vacuum Workstation. The marked positions contain 70% ethanol (1), PyroMark Denaturation Solution (2), PyroMark Wash Buffer (3), and high-purity water (4).

16. Apply vacuum to the tool by opening the vacuum switch on the vacuum workstation.

17. Wash the filter probes by lowering the probes into high-purity water (parking position).

Let approximately 180 ml of water flush through the filter probes; i.e., empty the trough.

18. Refill the parking position with 180 ml high-purity water.

19. Carefully lower the filter probes into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.

Note: Sepharose beads sediment quickly. If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.

20. Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.

21. Transfer the tool to the trough containing PyroMark Denaturation Solution (trough 2). Flush the filter probes for 5 s.

22. Transfer the tool to the trough containing PyroMark Wash Buffer (trough 3). Flush the filter probes for 10 s.

23. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes.



Figure 14. Illustration of the vacuum tool raised to beyond 90° vertical.

24. While the tool is held over the PyroMark Q96 Plate Low, close the vacuum switch on the vacuum workstation (Off).

25. Release the beads in the plate containing 40 μ l PyroMark Annealing Buffer by shaking the tool from side to side. Allow the filter probes to rest on the bottom of the wells.

26. Transfer the tool to the trough containing high-purity water (trough 4) and agitate the tool for 10 s.

27. Wash the filter probes by lowering the probes into the second trough with high-purity water (parking position) and applying vacuum. Flush the probes with 180 ml high-purity water.

28. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 14).
29. Turn off the vacuum switch on the vacuum workstation (Off), and place the tool in the Parking (P) position.
30. Turn off the vacuum pump.

At the end of a working day, liquid waste and remaining solutions should be discarded and the PyroMark Q96 Vacuum Workstation should be checked for dust and spillage, see Appendix C, page 75.
31. Heat the PyroMark Q96 Plate Low with the samples at 80°C for 2 min using a heating block and the prewarmed PyroMark Q96 Sample Prep Thermoplate Low.
32. Remove the PyroMark Q96 Plate from the thermo plate, and let the samples cool to room temperature (15–25°C) for at least 5 min.
33. Load the PyroMark Q96 Cartridge with the appropriate volumes of PyroMark Gold Q96 Reagents, as given in the **Pre Run Information** report from step 7.

Note: Make sure that no air bubbles are transferred from the pipet to the tips, and that all liquid is collected at the bottom of the cartridge. (See Troubleshooting Guide, page 64, for more information.)

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 the assay.
34. Switch on the instrument.
35. Open the instrument lid.
36. Open the process chamber lid.
37. Open the plate-holding frame.
38. Place the PyroMark Q96 Plate Low on the heating block.
39. Close the plate-holding frame and the process chamber lid.
40. Open the dispensing unit cover; release the latch, then open the cover.
41. Insert the filled dispensing cartridge with the label facing out.

42. Close the dispensing unit cover. Make sure that the latch snaps into its locked position.
43. Close the instrument lid.
44. Perform a run (see the *PyroMark Q96 ID User Manual*).
45. After run open the instrument lid.
46. Open the dispensing unit and remove the reagent cartridge by lifting it up and pulling it out.
47. Close the dispensing unit.
48. Open the process chamber lid and remove the PyroMark Q96 Plate Low from the heating block.
49. Close the process chamber and the instrument lid.
50. Discard the PyroMark Q96 Plate Low and clean the PyroMark Q96 Cartridge (see the *PyroMark Gold Q96 Reagents Handbook*).
51. Open the run in the PyroMark Q96 ID Software 2.5 and analyze all wells (see *PyroMark Q96 ID Software 2.5 Online Help*). The peak pattern should look like the one in Figure 15.

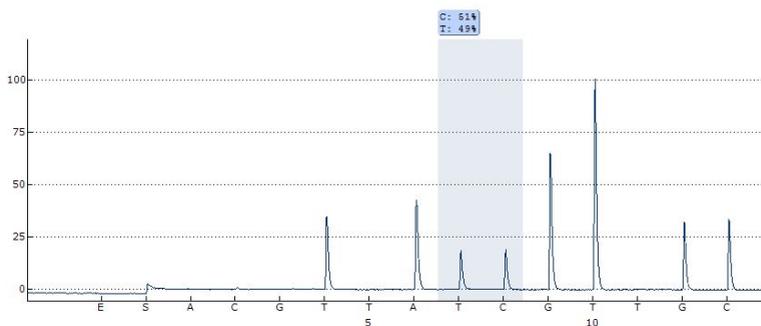


Figure 15.Pyrogram of the PyroMark Q96 ID Troubleshooting Run.

52. Confirm the proper installation of the system and use of the reagents by evaluating the quality assessment, quantification results, background and difference in peak height with and without sample preparation.

To obtain peak height values, select **Export Peak Heights** from the **Tools** menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft Excel (Delimited), and calculate the mean single peak height and background for each well as described below.

- **Perform a quality assessment.**

All wells should give "Passed" quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with % C indicated in a blue rectangle in the Pyrogram. If the quality assessment is "Check" or "Failed", look in **Well Information** for explanations.

- **Evaluate the quantification results.**

Select the **AQ Analysis Statistics Report** from the **Reports** menu. Quantification results are given in the report with standard deviation. The %C should be in the range 40–60%. The standard deviation should not exceed 2% units.

- **Evaluate single peak heights.**

The mean single peak height should be 35 ± 10 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 4, 6, 12, 13)}}{4}$$

- **Evaluate the background.**

Background from blank dispensations should not exceed 5%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 2, 3, 5)}}{\text{Sum single peaks (dispensation 4, 6, 12, 13)}} \times 100$$

- **Evaluate the difference in peak heights with and without sample preparation.**

The reduction in peak height between samples prepared using the PyroMark Q96 Vacuum Workstation compared with PyroMark Control Oligo added directly to the PyroMark Q96 Plate Low should not be more than 20%.

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see “Troubleshooting guide”, page 60, for possible causes and actions to be taken. If the troubleshooting guide does not explain the problem, 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).

Protocol: Troubleshooting Procedure for PyroMark Q96 MD together with the PyroMark Q96 Vacuum Workstation

If an unexpected result has been obtained it is crucial to determine whether this is related to PyroMark Q96 MD, the PyroMark Q96 Vacuum Workstation, or the assay. This protocol describes how to use PyroMark Control Oligo to verify the function of PyroMark Q96 MD, comparing results of samples prepared with or without the PyroMark Q96 Vacuum Workstation.

Important point before starting

- Assay and run files can be set up using either PyroMark Q96 MD Software or PyroMark CpG Software
- For further information on how to create an Assay Setup and a Run Setup, see the *PyroMark Q96 MD Software Online Help*.
- For further information on how to create an **Assay Setup** and a **Run Setup** with the PyroMark CpG software see the *PyroMark CpG Software Online Help*.

Things to do before starting

- Follow the instructions in *PyroMark Q96 MD User Manual* to install the PyroMark Q96 MD.
- The dilution buffer provided with the PyroMark Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Place the PyroMark Q96 HS Sample Prep Thermoplate on a heating block at 80°C for use in step 35.

- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. Set up a simplex entry for the PyroMark Control Oligo by using the PyroMark Q96 MD Software. Alternatively, set up an assay in the PyroMark CpG Software using the parameters below.
2. Type the following sequence in **Sequence to Analyze**: TAYGGTTTGCA.
3. Manually enter the following **Dispensation Order**: ACGTTATCGTTGC.

For more information on how to create an Assay Setup file, see the *PyroMark Q96 MD Software Online Help* or the *PyroMark CpG Software Online Help*.

Note: For the CpG assay, ignore the warning about sequence direction that is shown when the sequence to analyze and dispensation order are added.

For a thorough control of the instrument, sample preparation and reagents, additional blanks are included in the dispensing order.

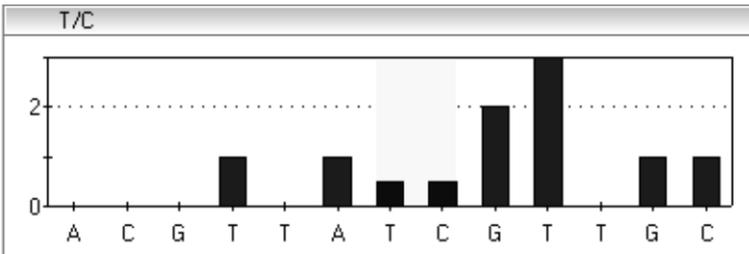


Figure 16. Histogram from PyroMark Q96 MD Software. Nucleotide additions 1, 2, 3, 5 and 11 are blank dispensations and serve as negative controls. The seventh and the eighth dispensations analyze the variable position (wobbled/degenerated base).

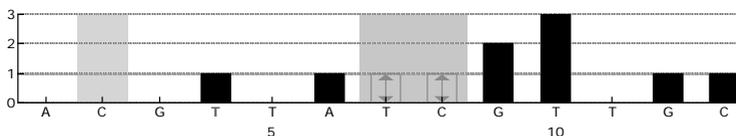


Figure 17. Histogram from PyroMark CpG Software. Nucleotide additions 1, 2, 3, 5 and 11 are blank dispensations and serve as negative controls. The seventh and the eighth dispensations analyze the variable position (wobbled/degenerated base).

4. Save the entry (PyroMark Q96 MD Software)/assay (PyroMark CpG Software).
5. Open a new run and allocate the created entry/assay to appropriate wells. Choose instrument parameters supplied by QIAGEN according to the reagents and cartridge that will be used for the run.

We recommend using 16 wells: 8 wells for samples prepared using the PyroMark Q96 Vacuum Workstation and 8 samples added directly to PyroMark Q96 HS Plate.

Note: A well is color-coded according to the assay type loaded to the well.

For more information on how to create a Run Setup file in PyroMark Q96 MD Software, see the *PyroMark Q96 MD Software Online Help*. For more information on how to create a Run Setup file in PyroMark CpG Software, see the *PyroMark CpG Software Online Help*.

6. Save the **Run Setup**.
7. Print a list of required volumes of enzyme mix, substrate mix, and nucleotides, and the plate setup.

Note: To generate a list of required volumes in the PyroMark Q96 MD Software click **View**, and **Run** in the browser area.

To generate a list of required volumes in the PyroMark CpG Software, open the run file and select **Volume Information** from the **Tools** menu.

8. Prepare a master mix for DNA immobilization according to Table 10. Check the lot number of the Streptavidin Sepharose HP. For lot number 10057037 or higher, use 1 μ l. For lot numbers lower than 10057037, use 2 μ l. Prepare a volume at least 10% greater than that required for the total number of reactions to be performed.

Note: Gently shake the bottle containing Streptavidin Sepharose High Performance until it is a homogeneous solution.

Table 10. 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 μ l	2 μ l
PyroMark Binding Buffer	40 μ l	40 μ l
High-purity water	27 μ l	26 μ l
Total volume	68 μl	68 μl

9. Dilute the PyroMark Control Oligo to 0.06 μ M as shown in Table 11.

Table 11. Dilution of the PyroMark Control Oligo

Component	Volume	Concentration
PyroMark Control Oligo	10 μ l	20 μ M
1x Dilution buffer*	90 μ l	–
First serial dilution	100 μ l	2 μ M
First serial dilution (from above)	10 μ l	2 μ M
1x Dilution buffer*	340 μ l	–
Final dilution	350 μ l	0.06 μ M

* Make sure that the 10x dilution buffer supplied with the PyroMark Control Oligo is diluted with high-purity water before use. See “Things to do before starting”, page 45.

10. Add 68 μ l of the master mix and 12 μ l of the diluted (0.06 μ M) PyroMark Control Oligo to 8 wells of a 96-well PCR plate or strips.

11. Seal the PCR plate (or strips) using strip caps.

12. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm.

Note: Sepharose beads sediment quickly. Capturing of beads must take place immediately after agitation.

During this step, prepare the PyroMark Q96 Vacuum Workstation for sample preparation (see Appendix B, page 73).

13. Add 12 μl of PyroMark Annealing Buffer to the wells of the PyroMark Q96 HS Plate that will be used with the immobilized PyroMark Control Oligo to be processed with the PyroMark Q96 Vacuum Workstation. Add 12 μl of the diluted (0.06 μM) PyroMark Control Oligo to 8 additional wells according to the **Run Setup**.

Note: Since the oligonucleotide is self-primed, no sequencing primer is required. The beads are released into PyroMark Annealing Buffer.

14. Place the PCR plate (or strips) and the PyroMark Q96 HS Plate on the worktable of the PyroMark Q96 Vacuum Workstation (see Figure 18).

Note: Ensure that the plate is in the same orientation as when samples were loaded.

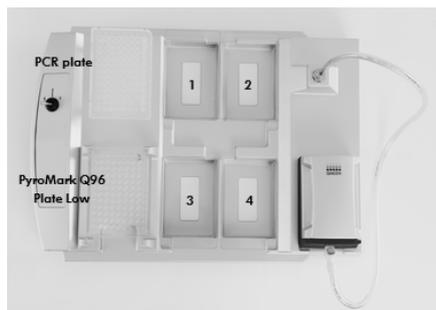


Figure 18. Placement of PCR plate (or strips) and PyroMark Q96 HS Plate on the PyroMark Q96 Vacuum Workstation. The marked positions contain 70% ethanol (1), PyroMark Denaturation Solution (2), PyroMark Wash Buffer (3), and high-purity water (4).

15. Apply vacuum to the tool by opening the vacuum switch on the vacuum workstation.
 16. Wash the filter probes by lowering the probes into high-purity water (parking position). Let approximately 180 ml of water flush through the filter probes; i.e., empty the trough.
 17. Refill the parking position with 180 ml high-purity water.
 18. Carefully lower the filter probes into the PCR plate (or strips) to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the tool.
- Note:** Sepharose beads sediment quickly. If more than 1 min has elapsed since the plate (or strips) was agitated, agitate again for 1 min before capturing the beads.

19. Transfer the tool to the trough containing 70% ethanol (trough 1). Flush the filter probes for 5 s.
20. Transfer the tool to the trough containing PyroMark Denaturation Solution (trough 2). Flush the filter probes for 5 s.
21. Transfer the tool to the trough containing PyroMark Wash Buffer (trough 3). Flush the filter probes for 10 s.
22. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes.



Figure 19. Illustration of the vacuum tool raised to beyond 90° vertical.

23. While the tool is held over the PyroMark Q96 HS Plate, close the vacuum switch on the vacuum workstation (Off).
24. Release the beads in the plate containing 12 μ l PyroMark Annealing Buffer by shaking the tool from side to side. Allow the filter probes to rest on the bottom of the wells.
25. Transfer the tool to the trough containing high-purity water (trough 4) and agitate the tool for 10 s.
26. Wash the filter probes by lowering the probes into the second trough with high-purity water (parking position) and applying vacuum. Flush the probes with 180 ml high-purity water.
27. Raise the tool up and back, beyond 90° vertical, for 5 s to drain liquid from the filter probes (see Figure 19).
28. Turn off the vacuum switch on the vacuum workstation (Off), and place the tool in the Parking (P) position.
29. Turn off the vacuum pump.

Note: At the end of a working day, liquid waste and remaining solutions should be discarded and the PyroMark Q96 Vacuum Workstation should be checked for dust and spillage, see Appendix C, page 75.

30. Heat the PyroMark Q96 HS Plate with the samples at 80°C for 2 min using a heating block and the prewarmed PyroMark HS Q96 Sample Prep Thermo Plate Kit.

Note: Use one Sample Prep Thermo Plate as lid on the plate to prevent evaporation of the samples.

31. Remove the PyroMark Q96 HS Plate from the thermo plate, and let the samples cool to room temperature (15–25°C) for at least 5 min.

32. Load the nucleotide and reagent tips in the PyroMark Q96 Dispensing Tip Holder with the appropriate volumes of PyroMark Gold Q96 Reagents.

Note: Make sure that no air bubbles are transferred from the pipet to the tips, and that all liquid is collected at the bottom of the cartridge. (See Troubleshooting Guide, page 64, for more information.)

Note: To generate a list of required volumes in the PyroMark Q96 MD Software see **View**, and **Run**.

To generate a list of required volumes in the PyroMark CpG Software, open the run file and select **Volume Information** from the **Tools** menu.

33. Switch on the instrument.

34. Open the process chamber lid using either software.

35. Place the PyroMark Q96 HS Plate on the heating block. Close the process chamber lid.

36. Open the dispensing unit cover by releasing the latch. With the two RDTs furthest away from you, insert the filled dispensing tip holder into position.

37. Close the dispensing unit cover. Make sure that the latch snaps into its locked position.

38. Close the instrument lid.

39. Perform a run (see *PyroMark Q96 MD User Manual* or *PyroMark CpG Software Online Help*).

40. After run open the instrument lid.

41. Open the dispensing unit and remove the dispensing tip holder and the PyroMark Q96 HS Plate.
42. Close the dispensing unit and the instrument lid (see *PyroMark Q96 MD User Manual* or *PyroMark CpG Software Online Help*).
43. Discard the PyroMark Q96 Plate and clean the tips in the PyroMark Q96 Dispensing Tip Holder (see the *PyroMark Gold Q96 Reagents Handbook*).
44. Open the run in the PyroMark Q96 MD Software and analyze all wells. The peak pattern should look like the one in Figure 20.

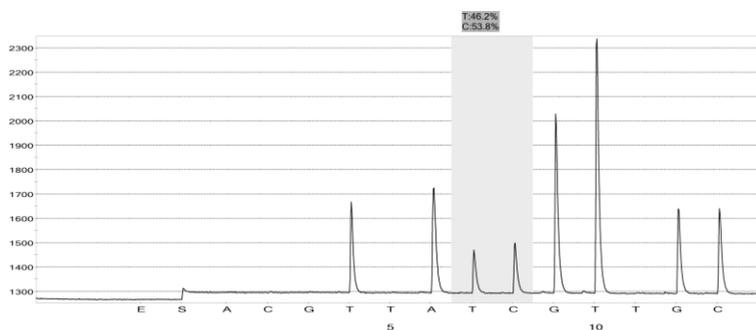


Figure 20. Pyrogram trace from PyroMark MD Software, AQ mode.

45. Confirm the proper installation of the system and use of the reagents by evaluating the quality assessment, quantification results, background and difference in peak height with and without sample preparation.

PyroMark Q96 MD Software: Select all wells by holding down the Ctrl or Shift key and click in the wells to mark them. Selected wells appear with a square around them. Click the arrow next to the **Peak Heights Button** on the **Peak Heights Tab**. Select **Export** from the drop-down list and save the data. Open this file in Microsoft Excel (Delimited) and calculate the mean single peak height and background for each well see below).

PyroMark CpG Software: Select **Peak Heights** from the **Reports** menu. Save the data in a suitable format (.csv or .tsv). Open this file in Microsoft Excel (Delimited) and calculate the mean single peak height and background for each well (see below).

- **Perform a quality assessment.**

All wells should give "Passed" quality. If the quality assessment is "Check" or "Failed", look in **Well info** (PyroMark Q96 MD Software) **Well Information** (PyroMark CpG Software) for explanations.

- **Evaluate the quantification results.**

PyroMark Q96 MD Software: Quantification results with standard deviation are shown in **AQ Statistics** under the **Statistics Tab**. Select all wells by holding down the **Ctrl** or **Shift** key and click in the wells one-by-one to mark them. Selected wells appear with a square around them. Click the **Statistics** button under the **Statistics Tab**.

PyroMark CpG Software: Quantification results with standard deviation are shown in the **CpG Statistics Report** that can be selected from the **Reports** menu. The %C should be in the range 40 to 60%. The standard deviation should not exceed 2% units.

- **Evaluate single peak heights.**

The mean single peak height should be ≥ 350 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 4, 6, 12, 13)}}{4}$$

- **Evaluate the background.**

Background from blank dispensations should not exceed 3%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 2, 3, 5)}}{\text{Sum single peaks (dispensation 4, 6, 12, 13)}} \times 100$$

-
- **Evaluate the difference in peak heights with and without sample preparation.**

The reduction in peak height between samples prepared using the PyroMark Q96 Vacuum Workstation compared with PyroMark Control Oligo added directly to the PyroMark Q96 HS Plate should not be more than 20%.

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see "Troubleshooting guide", page 60, for possible causes and actions to be taken. If the troubleshooting guide does not explain the problem, 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).

Protocol: Verifying the Function of PyroMark Q48 Autoprep

This protocol describes how to use the PyroMark Control Oligo to verify the function of PyroMark Q48 Autoprep.

Things to do before starting

- Follow the instructions in *PyroMark Q48 Autoprep User Manual* to install the PyroMark Q48.
- The dilution buffer provided with the PyroMark Control Oligo needs to be diluted before use. Prepare 1x dilution buffer by mixing 200 µl of 10x dilution buffer with 1800 µl of high-purity water.
- Allow all required reagents and solutions to reach room temperature (15–25°C) before starting.

Procedure

1. Set up an assay for the PyroMark Control Oligo in the PyroMark Q48 Software.
2. Click  in the toolbar and select **New AQ Assay**.
3. Type the following sequence in **Sequence to Analyze**: TAYGGTTTGC.
4. Click the **Generate Dispensation Order** icon to get the following nucleotide dispensation order: CTGACTGTG.

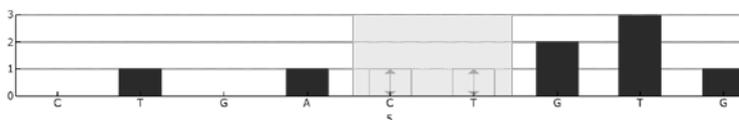


Figure 21. Histogram for AQ mode. Nucleotide additions 1 and 3 are blank dispensations and serve as negative controls. The fifth and the sixth dispensations analyze the variable position (wobbled/degenerated base).

5. Click  in the toolbar to save the assay.

6. Create a **Run Setup** by importing the assay parameters to 16 wells. Use four consecutive wells per disc section.
7. To add an assay to a well, you can either:
 - Right-click the well and select **Load Assay** from the context menu, or
 - Select the assay in the shortcut browser, and click and drag the assay to the well.A well is color-coded according to the assay type loaded to the well.
8. Select the **Standard** protocol in the protocol drop-down list.
9. **Recommended:** Select automatic primer loading in the **Run Setup**.

Note: PyroMark Advanced Annealing Buffer will be used instead of sequencing primer. When using automatic primer loading, one injector must be chosen, and the PyroMark Advanced Annealing Buffer will be loaded into the designated injector at the beginning of the run.

If you choose to use manual primer loading, you must pipet 2 μ l of PyroMark Advanced Annealing Buffer into each well during the sequencing run.
10. Save the **Run Setup** to a USB memory stick (supplied with PyroMark Q48 Autprep) and load onto the instrument. Alternatively, the run file can be transferred to the instrument via a network drive.
11. Start the sequencing run by selecting **Sequence** on the home screen and loading the run either via USB stick or network connection (selectable on the left side of the screen). Start the run setup by clicking on its name and the arrow that appears behind the name.
12. Insert the absorber strip into the absorber strip cavity by sliding it into place, ensuring that it sits level.
13. Load each reagent and nucleotide into the designated injector, according to the volumes shown on the instrument screen. Use the volumes displayed to ensure sufficient reagent is available for the initial injector prime as well as for dispensations during the run.

Note: Use PyroMark Advanced Annealing Buffer instead of primer.
14. Once all the reservoirs have been filled with the required reagent, close and lock the cartridge lids using the lid locks.

15. Select the **Start** button to begin injector priming and testing.
16. A confirmation message “Start prime and test?” will appear. To confirm select **Yes**.
Once injector priming has completed successfully, the software will progress to the next step with the chamber lid automatically opening.
17. Insert a PyroMark Q48 Disc into the instrument.
18. Lock the disc into position by screwing down the lock nut.
19. Dilute the PyroMark Control Oligo to 0.08 μM as shown in Table 12.

Note: 10 μl of the diluted (0.08 μM) PyroMark Control Oligo is needed per well.

Table 12. Dilution of the PyroMark Control Oligo

Component	Volume	Concentration
PyroMark Control Oligo	10 μl	20 μM
1x Dilution buffer*	90 μl	–
First serial dilution	100 μl	2 μM
First serial dilution (from above)	60 μl	2 μM
1x Dilution buffer*	1440 μl	–
Final dilution	1500 μl	0.08 μM

* Make sure that the 10x dilution buffer supplied with the PyroMark Control Oligo is diluted with high-purity water before use. See “Things to do before starting”, page 55.

† Provides a sufficient amount for 150 PyroMark Q48 test reactions.

20. Pipet 3 μl PyroMark Q48 Magnetic Beads into the required wells of the PyroMark Q48 Disc.
Note: PyroMark Q48 Magnetic Beads clump very quickly after mixing due to their large size. Please follow the guidelines described in the *PyroMark Q48 Autoprep User Manual* when pipetting the beads. Failure to do so will result in inconsistent bead amounts and poor sequencing results.
21. Pipet 10 μl of the diluted (0.08 μM) PyroMark Control Oligo from step 20 into each well of the PyroMark Q48 Disc.
22. Begin the run by selecting the **Start** button.

23. A confirmation message will display “Close injector cover. Start run?” To start the run close the injector cover and select **Yes**.

To go back and make any adjustments or to confirm any setting, select **No**.

24. If manual sequencing primer loading has been selected, the instrument will indicate that the sequence primer must be loaded, with an audible beep and display of the following message, “Select OK to open lid and add primers”.

Begin manual sequence primer loading by selecting the **OK** button.

The chamber lid will automatically open allowing the user to load the primers. Selecting **Cancel** will bring up a confirmation dialogue box, “Do you wish to cancel?” Selecting **Yes** will terminate the run.

Manually pipette 2 μ l of PyroMark Advanced Annealing Buffer into each well.

After the PyroMark Advanced Annealing Buffer has been loaded into the wells, select the **Next** button to proceed.

25. The chamber lid will close automatically and a countdown timer will display the time until sequencing. No further action is required from the user until the completion of the run.

26. Open the run in the PyroMark Q48 Software and analyze all wells. The peak pattern of the run should look like the one in Figure 22.

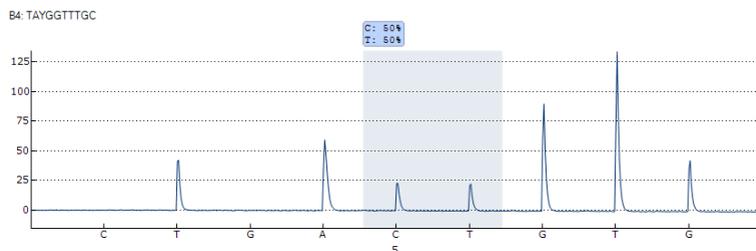


Figure 22. Example Run of the control Oligo on the PyroMark Q48 Autoprep.

To obtain peak height values, select **Export Peak Heights** from the **Tools** menu. Save the data in a suitable format (*.csv or *.tsv). Open this file in Microsoft Excel (Delimited), and calculate the mean single peak height for each well as described below.

- **Perform a quality assessment.**

All wells should give “Passed” quality, shown as a blue bar in the bottom field of the well when looking at the overview tab and with % C indicated in a blue rectangle in the Pyrogram. If the quality assessment is “Check” or “Failed”, look in **Well Information** for explanations.

- **Evaluate the quantification results.**

Select the **AQ Analysis Statistics Report** from the **Reports** menu. Quantification results are given in the report with standard deviation. The %C should be in the range 40–60%. The standard deviation should not exceed 2% units.

- **Evaluate single peak heights.**

The mean single peak height should be 70 ± 40 RLU.

$$\text{Mean single peak height} = \frac{\text{Sum single peaks (dispensation 2, 4, 9)}}{3}$$

- **Evaluate the background.**

Background from blank dispensations should not exceed 3%.

$$\text{Background (\%)} = \frac{\text{Sum blanks (dispensation 1, 3)} \div 2}{\text{Sum single peaks (dispensation 2, 4, 9)} \div 3} \times 100$$

If the values are within the set limits, the system is properly installed. If the results are not as stated above, see “Troubleshooting Guide”, page 60, for possible reasons for the failure and rerun the run. If the repeat of the run fails, 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).

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).

Sections are included for each evaluation performed:

- Quality assessment, page 61
- Quantification results, page 64
- Single peak heights, page 66
- Background, page 69
- Difference in peak height with and without sample preparation, page 69

Refer to the *PyroMark Q24 User Manual* for general troubleshooting of PyroMark Q24.

Refer to the *PyroMark Q24 Advanced User Manual* for general troubleshooting of PyroMark Q24 Advanced.

Refer to the *PyroMark Q96 ID User Manual* for general troubleshooting of PyroMark Q96 ID.

Refer to the *PyroMark Q96 MD User Manual* for general troubleshooting of PyroMark Q96 MD.

Refer to the *PyroMark Q48 Autoprep User Manual* for general troubleshooting of PyroMark Q48 Autoprep.

In the text below, the term "Dispensing Unit" includes Cartridges, NDTs, and CDTs used to dispense reagents in the various systems.

Quality assessment

Comments and suggestions

Warning from software about broad peaks

Concentration of PyroMark Control Oligo too high	Follow the relevant protocol. Make sure to dilute the PyroMark Control Oligo in dilution buffer as described in the protocols.
--------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------

High substrate peak

Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal; not applicable to PyroMark Q48 Autoprep)	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).
--------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Poor or incorrect sequence

- | | |
|------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) PyroMark Control Oligo not correctly prepared | Follow the instruction in the protocols for preparing the PyroMark Control Oligo. Make sure to dilute the PyroMark Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water. |
| b) Incorrect dispensation order | Check that the correct sequence was typed in the Assay Setup. |
| c) Buffers or reagents incorrectly diluted or incorrectly stored | Follow the instructions supplied with the reagents. Include an empty well (containing only PyroMark Annealing Buffer) in your run to check if background peaks are coming from the nucleotides. |
| d) Dispensation error (seen, for example, as split peaks) | Clean or replace the PyroMark Q24 or Q96 Cartridge. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com). |

Comments and suggestions

- | | |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| e) Blocked Dispensing Unit | Nucleotides are not dispensed correctly due to a blocked needle in the Dispensing Unit. Clean the Dispensing Unit and check that it is working properly. |
| f) Damaged Dispensing Unit | Discard the Dispensing Unit according to federal, state, and local environmental regulations for disposal of laboratory waste. |
| g) Annealing time too long | Carry out annealing for the correct time and at the temperatures described in the protocols. |

Small or missing peaks

- | | |
|------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Insufficient amount of template for immobilization | Make sure to dilute the PyroMark Control Oligo correctly and use the amounts specified in the protocols. |
| b) Not enough enzyme or substrate for all wells | Fill the Dispensing Unit according to the instructions in the Pre Run Information report. |
| c) Wells marked in the Run Setup do not align with sample placement in the plate | Check that you loaded the PyroMark Plate correctly, according to the Run Setup. |
| d) One or more of the nucleotide compartments in the Dispensing Unit not correctly filled with reagents or nucleotides | Make sure that sufficient reagents are added to the Dispensing Unit. Follow the instructions for use supplied with the products. |
| e) Dispensation error (seen, for example, as split peaks) | Clean or replace the Dispensing Unit. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com). |

Comments and suggestions

- | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| f) Blocked Dispensing Unit | Nucleotides are not dispensed correctly due to a blocked needle in the Dispensing Unit. Clean the Dispensing Unit and check that it is working properly.
Enzymes or substrates are not dispensed correctly due to a blocked Dispensing Unit (as indicated by a missing presequencing signal and no peaks in the Pyrogram). Clean the Dispensing Unit and check that it is working properly.
Note: The presequencing signal is not applicable for PyroMark Q48 Autoprep. |
| g) Damaged Dispensing Unit | Discard the Dispensing Unit according to federal, state, and local environmental regulations for disposal of laboratory waste. |
| h) Buffers or reagents incorrectly diluted or incorrectly stored | Follow the instructions supplied with the reagents. |
| i) PyroMark instrument started without a plate inserted | Clean the heating block and the light guides/lens array according to instructions in the user manual for the instrument. |
| j) PyroMark Control Oligo not correctly prepared | Follow the instruction in the protocols for preparing the PyroMark Control Oligo. Make sure to dilute the PyroMark Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water. |
| k) Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal; not applicable for PyroMark Q48 Autoprep) | 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). |

Comments and suggestions

- l) Air bubbles are transferred from the pipet to the cartridge
- Air bubbles in the cartridge well will prevent the liquid from being dispensed during the Pyrosequencing run. Air bubbles can be removed 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.
- m) PyroMark reagents and nucleotides adhere to the wall of the cartridge
- If pipetting small volumes of reagents into the cartridge (e.g., below 50 μ l 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 liquid used can be increased.

Warning regarding signal to noise

Various

See points a) to k) in "Small or missing peaks", above.

Quantification results

Comments and suggestions

Poor or incorrect sequence

- a) PyroMark Control Oligo not correctly prepared
- Follow the instruction in the protocols for preparing the PyroMark Control Oligo. Make sure to dilute the PyroMark Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
- b) Incorrect sequence to analyze or dispensation order
- Check that the correct sequence was typed in the Assay Setup.

Comments and suggestions

- | | |
|------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| c) Buffers or reagents incorrectly diluted or incorrectly stored | Follow the instructions supplied with the reagents. Include an empty well (containing only PyroMark Annealing Buffer) in your run to check if background peaks are coming from the nucleotides. |
| d) Dispensation error (seen, for example, as split peaks) | Clean or replace the Dispensing Unit. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com). |
| e) Blocked Dispensing Unit | Nucleotides are not dispensed correctly due to a blocked needle in the Dispensing Unit. Clean the Dispensing Unit and check that it is working properly. |
| f) Damaged Dispensing Unit | Discard the Dispensing Unit according to federal, state, and local environmental regulations for disposal of laboratory waste. |
| g) Annealing time too long | Carry out annealing for the correct time and at the temperatures described in the protocols. |

High background

- | | |
|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| a) The storage conditions for one or more reagent did not comply with the instructions given in "Storage", page 4. | Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary. |
| b) Reagents have expired | Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary. |

Single peak heights

Comments and suggestions

Poor or incorrect sequence

- | | |
|------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) PyroMark Control Oligo not correctly prepared | Follow the instruction in the protocols for preparing the PyroMark Control Oligo. Make sure to dilute the PyroMark Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water. |
| b) Incorrect sequence to analyze or dispensation order | Check that the correct sequence was typed in the Assay Setup. |
| c) Buffers or reagents incorrectly diluted or incorrectly stored | Follow the instructions supplied with the reagents. Include an empty well (containing only PyroMark Annealing Buffer) in your run to check if background peaks are coming from the nucleotides. |
| d) Dispensation error (seen, for example, as split peaks) | Clean or replace the Dispensing Unit. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com). |
| e) Blocked PyroMark Dispensing Unit | Nucleotides are not dispensed correctly due to a blocked needle in the Dispensing Unit. Clean the Dispensing Unit and check that it is working properly. |
| f) Damaged PyroMark Dispensing Unit | Discard the Dispensing Unit according to federal, state, and local environmental regulations for disposal of laboratory waste. |
| g) Annealing time too long | Carry out annealing for the correct time and at the temperatures described in the protocols. |

Comments and suggestions

Small or missing peaks

- | | |
|------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| a) Insufficient amount of template for immobilization | Make sure to dilute the PyroMark Control Oligo correctly and use the amounts specified in the protocols. |
| b) Not enough enzyme or substrate for all wells | Fill the Dispensing Unit according to the instructions in the Pre Run Information report. |
| c) Wells marked in the Run Setup do not align with sample placement in the plate | Check that you loaded the PyroMark Q24 or Q96 Plate correctly, according to the Run Setup. |
| d) One or more of the nucleotide compartments in the Dispensing Unit not correctly filled with reagents or nucleotides | Make sure that sufficient reagents are added to the Dispensing Unit. Follow the instructions for use supplied with the products. |
| e) Dispensation error (seen, for example, as split peaks) | Clean or replace the Dispensing Unit. If the problem remains, contact QIAGEN Technical Services (for contact information, see back cover or visit www.qiagen.com). |
| f) Blocked Dispensing Unit | <p>Nucleotides are not dispensed correctly due to a blocked needle in the Dispensing Unit. Clean the Dispensing Unit and check that it is working properly.</p> <p>Enzymes or substrates are not dispensed correctly due to a blocked Dispensing Unit (as indicated by a missing presequencing signal and no peaks in the Pyrogram). Clean the Dispensing Unit and check that it is working properly.</p> <p>Note: The presequencing signal is not applicable for PyroMark Q48 Autoprep.</p> |
| g) Damaged Dispensing Unit | Discard the Dispensing Unit according to federal, state, and local environmental regulations for disposal of laboratory waste. |

Comments and suggestions

- h) Buffers or reagents incorrectly diluted or incorrectly stored Follow the instructions supplied with the reagents.
- i) PyroMark Control Oligo not correctly prepared Follow the instruction in the protocols for preparing the PyroMark Control Oligo. Make sure to dilute the PyroMark Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.
- j) Contaminated sample leads to unusually high consumption of substrate mixture (noted as a high presequencing signal; not applicable to PyroMark Q48 Autoprep) 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).
- k) Air bubbles are transferred from the pipet to the cartridge Air bubbles in the cartridge well will prevent the liquid from being dispensed during the Pyrosequencing run. Air bubbles can be removed 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.
- l) PyroMark reagents and nucleotides adhere to the wall of the cartridge If pipetting small volumes of reagents into the cartridge (e.g., below 50 μ l 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 liquid used can be increased.

Comments and suggestions

Very high peaks

PyroMark Control Oligo not correctly prepared

Follow the instruction in the protocols for preparing the PyroMark Control Oligo. Make sure to dilute the PyroMark Control Oligo in the dilution buffer as described in the protocols. Make sure that the 10x dilution buffer provided is first diluted to 1x using high-purity water.

Background

Comments and suggestions

High background

a) The storage conditions for one or more reagent did not comply with the instructions given in "Storage", page 4.

Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary.

b) Reagents have expired

Check the storage conditions and the expiration date of the reagents and use new reagents, if necessary.

Difference in peak height with and without sample preparation

Comments and suggestions

Incorrect sample preparation

a) Liquid left in some wells or tubes when capturing the beads containing immobilized template to the filter probes

Replace corresponding filter probe in the vacuum tool of the PyroMark Q24 or PyroMark Q96 Vacuum Workstation. See the sample preparation guidelines available at 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).

Comments and suggestions

- | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| b) Filter probes not working properly | Check the filter probes. Add 80 μ l of high-purity water to each well of a PCR plate. Start the vacuum pump and apply vacuum by opening the vacuum switch (On). Lower the vacuum tool into the PCR plate and wait 10 s. Check that all wells of the PCR plate are empty. If not, replace the failed filter probes and repeat the test. |
| c) White debris (Streptavidin Sepharose High Performance beads) in some wells or tubes when capturing the beads containing immobilized template to the filter probes | Do not leave the PCR plate, used in the immobilization step, for longer than 1 min after mixing is finished. If necessary, mix for an extra minute before capturing the beads. |
| d) Leakage of the PyroMark Q24 or Q96 Vacuum Workstation | Ensure that the tubing is connected properly and that there is no leakage. The waste filter might be wet and need to be replaced. |

Appendix A: Preparation of the PyroMark Q24 Vacuum Workstation

This protocol is a description of how to prepare the PyroMark Q24 Vacuum Workstation before using it for preparation of single-stranded DNA.

Procedure

1. Fill 5 separate troughs (supplied with the PyroMark Q24 Vacuum Workstation) as follows.
 - Approximately 50 ml ethanol (70%) (1)
 - Approximately 40 ml PyroMark Denaturation Solution (2)
 - Approximately 50 ml PyroMark Wash Buffer (3)
 - Approximately 50 ml high-purity water (4)
 - Approximately 70 ml high-purity water (5)

A suggested setup is shown in Figure 23. Refill the troughs to these levels whenever necessary.



Figure 23. Positions on the PyroMark Q24 Vacuum Workstation.

2. Switch on the vacuum pump.
3. Apply vacuum to the tool by opening the vacuum switch.

-
4. Wash the filter probes by lowering the probes into high-purity water (trough 5). Flush the probes with 70 ml high-purity water. Make sure that the water is being transferred to the waste container. If it is not, then make sure that the tubing is connected correctly and is not broken. Broken tubing should be replaced, see “Replacing the tubing” in the *PyroMark Q24 User Manual*.
 5. Make sure that the waste filter is dry. If the filter is wet, it should be replaced, see “Replacing the waste filter” in the *PyroMark Q24 User Manual*.
 6. Refill trough 5 with 70 ml high-purity water.
 7. Close the vacuum switch on the tool (Off) and place the tool in the Parking (P) position.

Appendix B: Preparation of the PyroMark Q96 Vacuum Workstation

This protocol is a description of how to prepare the PyroMark Q96 Vacuum Workstation before using it for preparation of single-stranded DNA.

1. Fill 5 separate troughs (supplied with the PyroMark Q96 Vacuum Workstation) as follows.

- Approximately 110 ml ethanol (70%) (1)
- Approximately 90 ml PyroMark Denaturation Solution (2)
- Approximately 110 ml PyroMark Wash Buffer (3)
- Approximately 110 ml high-purity water (4)
- Approximately 180 ml high-purity water (Parking position)

A suggested setup is shown in Figure 24. Refill the troughs to these levels whenever necessary.

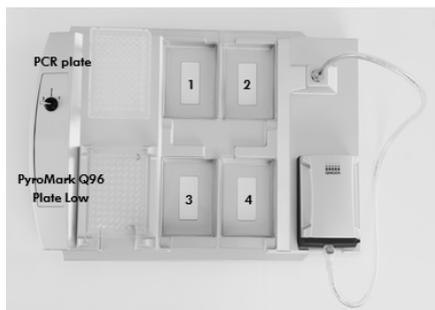


Figure 24. Placement of PCR plate (or strips) and PyroMark Q96 Plate Low or PyroMark Q96 HS Plate on the PyroMark Q96 Vacuum Workstation. The marked positions contain 70% ethanol (1), PyroMark Denaturation Solution (2), PyroMark Wash Buffer (3), and high-purity water (4).

2. Refill the troughs to approximately these levels whenever needed.
3. Start the vacuum pump.
4. Apply vacuum to the tool by opening the vacuum switch.
5. Wash the filter probes by lowering the probes into high purity water (Parking position). Let approximately 180 ml of water flush through the filter probes, i.e., empty the trough.

Ensure that the water is being transferred to the waste container. If not ensure that the tubing is connected properly and there is no leakage.

6. Refill Parking Position with 180 ml of high-purity water.
7. Switch off the vacuum switch on the vacuum workstation (Off) and place the tool in the Parking (P) position.

Appendix C: Emptying the Waste Container and Troughs

<p>WARNING</p> 	<p>Hazardous chemicals</p> <p>The PyroMark Denaturation Solution used with the PyroMark Q24 Vacuum Workstation contains sodium hydroxide, which is irritating to eyes and skin. 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.</p>
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* 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).

Be sure to observe federal, state and local environmental regulations for the disposal of laboratory waste.

The following item is required:

- High-purity water (Milli-Q 18.2 M Ω x cm, www.millipore.com, or equivalent).

Procedure

1. Ensure that no vacuum is applied to the vacuum tool, the vacuum switch is closed (Off), and the vacuum pump is switched off.
2. Discard any solutions left in the troughs.

-
3. Rinse the troughs with high-purity water, or replace them, if necessary.
 4. Empty the waste container.
 5. The cap can be removed without disconnecting the tubing.
 6. If the PyroMark Q24 Vacuum Workstation must be cleaned (for dust or spillage), follow the instructions in “Cleaning the PyroMark Q24 Vacuum Workstation” in the *PyroMark Q24 User Manual*.

Ordering Information

Product	Contents	Cat. no.
PyroMark Control Oligo	For installation check of PyroMark systems	979203
Instruments		
PyroMark Q24	Instrument and software for Pyrosequencing of 24 samples in parallel	9001514
PyroMark Q24 Advanced	Instrument and software for advanced Pyrosequencing of 24 samples in parallel	9002270
PyroMark Q24 Vacuum Workstation	Workstation for preparing single-stranded DNA from 24 samples	Varies*
PyroMark Q48 Autoprep	Instrument, software, and pipet for advanced Pyrosequencing of 48 samples in parallel	9002470
PyroMark Q96 ID	Sequence-based detection platform for Pyrosequencing of 96 samples in parallel	9001525
PyroMark Q96 Vacuum Workstation	Workstation for preparing single-stranded DNA from 96 samples; for use with PyroMark Q96 ID	Varies†
Consumables		
PyroMark Q24 Cartridge (3)	Cartridges for dispensing nucleotides and reagents	979202
PyroMark Q24 Plate (100)	24-well sequencing reaction plate	979201
PyroMark Q48 Discs (50)	50 discs for running PyroMark Q48 Autoprep reactions	974901
PyroMark Q48 Absorber Strips (100)	100 absorber strips for running PyroMark Q48 Autoprep reactions	974912

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

† 9001529 (220V); 9001528 (110V); 9001740 (100V).

Product	Contents	Cat. no.
PyroMark Q96 Cartridge	Cartridges for dispensing nucleotides and reagents	979004
PyroMark Q96 Plate Low (100)	96-well sequencing reaction plate, for use with PyroMark Q96 ID, 100 plates in each package	979002
Reagents and buffers		
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
PyroMark PCR Kit (200)*	PCR Master Mix for PCR reactions optimized for Pyrosequencing analysis	978703
PyroMark OneStep RT-PCR Kit (50)*	OneStep RT-PCR Enzyme Mix for RT-PCR reactions optimized for Pyrosequencing analysis	978801
PyroMark Gold Q24 Reagents (5 x 24)	Reagents for 5 x 24 PyroMark Q24 reactions	970802
PyroMark Q24 Advanced Reagents (4 x 24)	Reagents for 4 x 24 PyroMark Q24 Advanced standard reactions	970902
PyroMark Q24 Advanced CpG Reagents (4 x 24)	Reagents for 4 x 24 PyroMark Q24 Advanced CpG and long-read reactions	970922
PyroMark Q48 Advanced Reagents (4 x 48)	Reagents for 4 x 48 PyroMark Q48 Autoprep standard reactions	974002
PyroMark Q48 Advanced CpG Reagents (4 x 48)	Reagents for 4 x 48 PyroMark Q48 Autoprep CpG and long-read reactions	974022

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

Product	Contents	Cat. no.
PyroMark Q48 Autoprep Starter Kit	PyroMark Q48 Magnetic Beads (300), PyroMark Q48 Advanced CpG Reagents (4 x 48), PyroMark Control Oligo, PyroMark Q48 Discs (50) and PyroMark Q48 Absorber Strips (100)	974230
PyroMark Q48 Magnetic Beads (300)	Magnetic streptavidin-coated Sepharose beads for running 300 PyroMark Q48 Autoprep reactions	974203
PyroMark Gold Q96 Reagents (5 x 96)	Reagents for 5 x 96 Pyrosequencing reactions on the PyroMark Q96 ID	972804
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 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
EpiTect Fast DNA Bisulfite Kit (50)§	For 50 preps: Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59824
EpiTect Fast FFPE Bisulfite Kit (50)§	For 50 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59844
EpiTect Fast LyseAll Bisulfite Kit (50)§	For 50 preps: Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59864

* 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.

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

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Trademarks: QIAGEN[®], EpiTect[®], PyroMark[®], Pyrosequencing[®], Pyrogram[®], Sample to Insight[®] (QIAGEN Group); Excel[®], Microsoft[®] (Microsoft Corporation); Milli-Q[®] (Millipore Corporation); Sepharose[®] (GE Healthcare).

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the PyroMark Control Oligo to the following terms:

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Notes

Notes

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Technical Support

www.support.qiagen.com