

PyroMark[®] Q48 Advanced Reagents and PyroMark Q48 Advanced CpG Reagents

Store the PyroMark Q48 Advanced Reagents (cat. no. 974002) and PyroMark Q48 Advanced CpG Reagents (cat. no. 974022) at 2–8°C upon arrival. After reconstitution, the Enzyme Mixture and Substrate Mixture are stable for at least 5 days at 2–8°C, or can be frozen and stored in their vials at –20°C. Frozen reagents should not be subjected to more than 6 freeze–thaw cycles. Protect the Substrate Mixture from light. We recommend using PyroMark Q48 Advanced CpG Reagents for long-read Pyrosequencing[®] runs, such as *de novo* sequencing or methylation analysis, which may require larger volumes of nucleotides.

Further information

- *PyroMark Q48 Advanced Reagents and PyroMark Q48 Advanced CpG Reagents Handbook*: www.qiagen.com/HB-1901
- *PyroMark Q48 Autoprep User Manual*: www.qiagen.com/HB-1971
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- **Important**: Do not freeze the nucleotides.
- Dissolve the lyophilized Enzyme and Substrate Mixtures in 660 µl each of PyroMark Advanced Annealing Buffer (included in the kit). Mix by swirling the vial gently. Do not vortex! In order to ensure that the mixture is fully dissolved, leave it at room temperature (15–25°C) for 5–10 min. Make sure that the solution is not turbid before filling the PyroMark Q48 Cartridge. If the reagents are not to be used immediately, place the reagent vials on ice or in a refrigerator.

Equipment and reagents to be supplied by user

- Pipets (adjustable)
- Sterile pipet tips (with filters for PCR setup)
- PyroMark Q48 Magnetic Beads (cat. no. 974203)
- PyroMark Q48 Autoprep instrument (cat. no. 9002470, including PyroMark Q48 Cartridges and PyroMark Q48 Autoprep software)
- PyroMark Q48 Discs (cat. no. 974901)
- PyroMark Q48 Absorber Strips (cat.no. 974912)
- Sequencing primer (final concentration 4 μM)
- High-purity water (Milli-Q 18.2 $\text{M}\Omega \times \text{cm}$ or equivalent)

Preparing templates and reagents

At the beginning of the run, the instrument will guide you through run preparation, including absorber strip insertion, injector loading, disc insertion and bead and template loading.

To start a sequencing run, select **Sequence** on the home screen and load 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 behind the name.

Insertion of absorber strip

Insert the absorber strip into the absorber strip cavity by sliding it into place. Ensure that it sits level and that the ends meet on the left side of the absorber strip cavity (9 o'clock position).

Loading and priming of cartridges

1. Open the injector cover and then open each individual cartridge lid.
 2. Select injectors to prime by touching on the injector box. Selected injectors will display a droplet symbol (💧).
 3. Select if this is the last run of the day.
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4. Pipet each reagent into the designated injector, according to the volumes shown on the instrument touchscreen. Ensure that the tip of the pipette does not touch the filter at the bottom. Try to avoid air bubbles in the liquid.
 5. Once all the reservoirs have been filled with the required reagent, close and lock the cartridge lids using the lid locks.
 6. Click the **Start** button to begin injector priming and testing.
 7. A confirmation message "Start prime and test?" will appear. To confirm, select **Yes**.

All selected injectors will undergo the priming protocol. Following the prime, all required injectors will undergo a number of test shots. Injectors that pass the test will be displayed with a green tick (✓).

Once injector priming has completed successfully, the software will progress to the next step with the chamber lid automatically opening.

Inserting the PyroMark Q48 Disc into the instrument

1. Insert a PyroMark Q48 Disc into the instrument. Hold the disc using the disc grips, position the disc index hole (outer hole near well B1) over the disc index pin on the rotor hub, and insert.
2. The orientation of the disc grips should match up to those on the rotor. The disc detection system will recognize and warn the user if a disc is not properly inserted.
3. Lock the disc into position by screwing down the lock nut.
Important: The disc must be locked into position to avoid spilling the contents of the disc into the chamber.

Loading PyroMark Q48 Magnetic Beads into the PyroMark Q48 Disc

PyroMark Q48 Magnetic Beads clump very quickly after mixing due to their large size. Please follow the guidelines 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. Use the supplied electronic multi-dispense pipet when dispensing beads into a larger number of wells (>5). Never dispense beads into more than 5 wells at a time.

1. Vortex the bead slurry to homogeneity before dispensing.
2. Pipet 3 μl PyroMark Q48 Magnetic Beads into the required wells of the PyroMark Q48 Disc immediately after resuspension.
3. Repeat the resuspension step between each pipetting step.
4. Store the beads immediately after use according to the manufacturer's guidelines to ensure a longer shelf life.

Loading Pyrosequencing template into the PyroMark Q48 Disc

1. Pipet 10 μl biotinylated PCR product into the correct wells of the PyroMark Q48 Disc. The software indicates the well position for each sample. Alternatively, use the Pre Run printout from the PyroMark Q48 Autoprep Software as a sample position guide.
Note: If using less than 10 μl of PCR product, add high-purity water to the sample to give a final volume of 10 μl .
Note: When running the P4 extra protocol, the user will be prompted by the instrument to additionally pipet 5 μl PyroMark Binding Buffer into each well.
2. Ensure that the template thoroughly covers the beads inside the well.
3. Ensure that the disc is correctly inserted into the instrument and locked in place.

Starting a run

1. After the template has been loaded, begin the run by selecting the **Start** button.
2. 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**.



Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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