Quick-Start Protocol March 2016

PyroMark® Q24 Advanced Reagents – Part 1

Store the PyroMark Q24 Advanced Reagents (cat. no. 970902) 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.

Further information

- *PyroMark Q24 Advanced Reagents Handbook*: **www.qiagen.com/HB-1433**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- **Important**: Do not freeze the nucleotides
- These protocols describe immobilization of biotinylated PCR product to beads and loading the PyroMark Q24 Cartridge with reagents. See *Quick-Start Protocol: PyroMark Q24 Advanced Reagents – Part 2* for preparation of single-stranded template and annealing to primer, and starting the run.
- Switch on the PyroMark Q24 Advanced instrument at least 30 minutes before starting a run
- Place a PyroMark Q24 Plate Holder on a pre-heated heating block at 80°C
- Prepare the PyroMark Q24 Vacuum Workstation for sample preparation
- Dissolve the lyophilized Enzyme and Substrate Mixtures in 660 µl each of high-purity water (Milli-Q® 18.2 MΩ x cm or equivalent, filtered through a 0.22 µm filter). Mix by swirling the vial gently. Do not vortex! In order to ensure that the mixture is fully dissolved, leave it at room temperature (15–25°C) for 5–10 min. Make sure that the solution is not turbid before filling the PyroMark Q24 Cartridge. If the reagents are not to be used immediately, place the reagent vials on ice or in a refrigerator.

Equipment and reagents to be supplied by user

- Pipets (adjustable)
- Sterile pipet tips (with filters for PCR setup)
- Streptavidin Sepharose® High Performance (HP) beads (GE Healthcare, cat. no. 17-5113-01)
- PyroMark Q24 Advanced instrument
- PyroMark Q24 Advanced software
- PyroMark Q24 Plate (cat. no. 979201)
- PyroMark Q24 Cartridge (cat. no. 979202)
- PyroMark Q24 Vacuum Workstation
- Plate mixer for immobilization to beads
- Sequencing primer
- Heating block capable of attaining 80°C
- 24-well PCR plate or strips
- High-purity water (Milli-Q 18.2 MΩ x cm or equivalent)
- Ethanol (70%)
- PyroMark Wash Buffer
- PyroMark Denaturation Buffer
- Adhesive foil or strip caps

Protocol 1: Immobilization of PCR product to Sepharose beads

1. Gently shake the bottle containing Streptavidin Sepharose HP from side to side until it is a homogeneous solution.

   Note: Sepharose beads sediment quickly. Before pipetting, ensure the homogeneity of the bead solution by mixing.

2. Prepare the DNA immobilization reactions in a 24-well PCR plate according to Table 1 and your run setup in the PyroMark Q24 Advanced software.

Optional: When the amount of PCR product is the same for each reaction, prepare a master mix for DNA immobilization according to Table 1, with all components except the PCR product. Do not centrifuge the master mix.

Table 1. Reaction setup for DNA immobilization

	Volume per reaction	
Component	**For lot number 10057037 or higher**	**For lot numbers lower than 10057037**
Streptavidin Sepharose HP*	1 µl	2 µl
PyroMark Binding Buffer	40 µl	40 µl
High-purity water	Variable†	Variable†
Biotinylated PCR product	5–20 µl	5–20 µl
Total volume	**80 µl**	**80 µl**

* Check the lot number of the Streptavidin Sepharose HP. For lot number 10057037 or higher, use 1 µl. For lot numbers lower than 10057037, use 2 µl.

† The volume of water depends on the amount of PCR product used. For example, if using 15 µl of PCR product, 1 µl of beads, and 40 µl of Binding Buffer, add 24 µl of high-purity water.

3. Seal the PCR plate with adhesive foil. Ensure that no leakage is possible between the wells.
4. Agitate the PCR plate at room temperature (15–25°C) for 5–10 min at 1400 rpm. During this step, proceed immediately with "Protocol 2: Loading reagents into the PyroMark Q24 Cartridge".

Protocol 2: Loading reagents into the PyroMark Q24 Cartridge

1. Make sure that the lyophilized Enzyme Mixture and Substrate Mixture have been properly reconstituted (see "Notes before starting").
2. Allow the reagents and the PyroMark Q24 Cartridge to reach room temperature (15–25°C).
3. Place the PyroMark Q24 Cartridge with the label facing you.
4. Load the PyroMark Q24 Cartridge with the appropriate volumes of PyroMark Advanced nucleotides, Enzyme Mixture, and Substrate Mixture according to Figure 1.

   Note: The reagent volumes needed for a specific run are provided by the PyroMark Q24 Advanced software. After setting up the run, click **Tools** and choose **Pre Run Information** to see these volumes.

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.

Note: Use disposable tips without hydrophobic filters for loading the cartridge to permit correct function of the cartridge. Make sure that no air bubbles are transferred from the pipet to the cartridge.

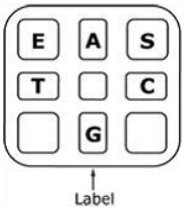

Figure 1. Illustration of the PyroMark Q24 Cartridge as seen from above. The annotations correspond to the label on the reagent vials. Add Enzyme Mixture (**E**), Substrate Mixture (**S**), and nucleotides (**A**, **T**, **C**, **G**) according to the volume information given in the **Pre Run Information report**, found in the **Tools** menu at run setup.

5. Open the cartridge gate on the PyroMark Q24 Advanced instrument and insert the filled reagent cartridge with the label facing out. Push the cartridge in fully and then push it down.
6. Ensure the line is visible in front of the cartridge and close the gate.
7. Proceed directly with "Protocol 3: Preparation of template DNA and annealing to primer" in *Quick-Start Protocol: PyroMark Q24 Advanced Reagents – Part 2*.

Scan QR code for handbook.

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