

Product Profile

PyroMark® Q48 Autoprep

Automated Pyrosequencing® with integrated template preparation for advanced methylation, mutation and SNP quantification

PyroMark Q48 Autoprep integrates fully automated template preparation into the Pyrosequencing protocol. The new design and software doubles the throughput of PyroMark Q24 Advanced, while keeping the improved performance, making it ideally suited for functional studies, as well as for verification and validation of large numbers of samples from NGS and array experiments.

PyroMark Q48 Autoprep provides:

- Less manual interaction – fully automated protocol with integrated template preparation
- Easy handling – intuitive instrument and analysis software for convenient control
- Higher throughput – more samples per run, more runs per day
- Best Pyrosequencing performance – advanced technology for long and reliable sequence runs
- More insight in DNA methylation analysis – quantitative analysis at consecutive CpG and CpN sites
- More SNP and mutation assays per run – multiple primer dispensation increases sample throughput

Automated protocol with integrated template preparation reduces manual user interaction

PyroMark Q48 Autoprep is a small benchtop instrument controlled by intuitive software and a large touchscreen. All required protocol steps are shown on the display and guide the user through the entire Pyrosequencing protocol, simplifying user interaction. The entire protocol is automated after loading of samples, magnetic beads and reagents. Template preparation and sequencing primer annealing are carried out by the instrument, with no further manual interaction from the user (Figure 2).

Optionally, sequencing primer addition can be performed manually to increase the number of different assays per run. In addition Multiple Primer Dispensation (MPD) can be used

to automate sequencing primer dispensation for an even higher number of assays per run.



Figure 1. PyroMark Q48 Autoprep.

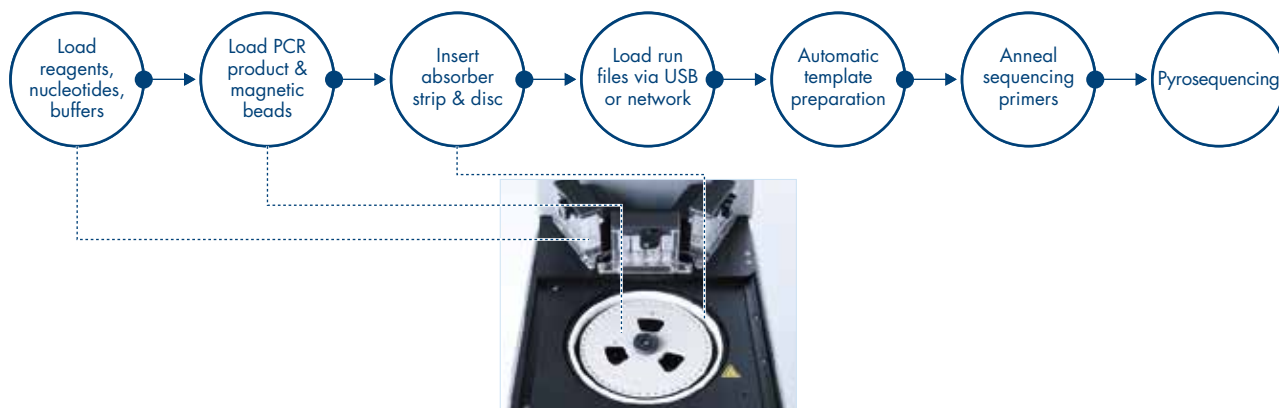


Figure 2. PyroMark Q48 Autoprep workflow with automated protocol and integrated template preparation. Three cartridges, each with 4 reservoirs, supply reagents, nucleotides, sequencing primers and buffers for automated template preparation and Pyrosequencing. After filling the cartridges and loading the disc with samples and magnetic beads, the disc and an absorber strip are placed into the instrument. Run files are loaded via USB stick or network connection, and the run is started. Up to 3 separate sequencing primers or MPD mixes can be dispensed automatically. Optional, manual primer addition increases the number of different assays per run.

Increased sample throughput for more SNP and mutation assays per run

Multiple Primer Dispensation (MPD) is a strategy to increase the capacity of automated sequencing primer dispensation in case more assays are needed than cartridge reservoirs are available. Using MPD, automatic sequencing primer

dispensation can be increased by a factor of 4 or even more, providing a fully automated workflow for 12 or more sequencing primers per run (see Figure 3).

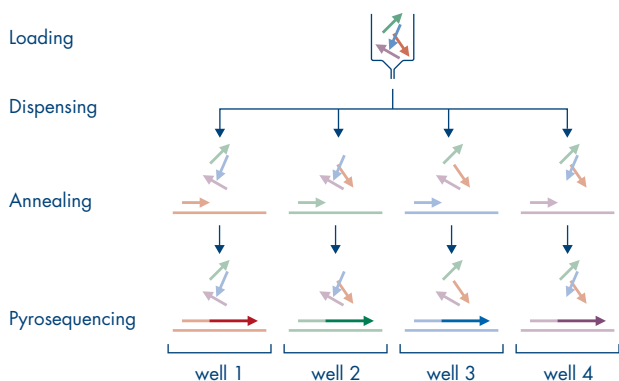


Figure 3. The principle of multiple primer dispensation. A sequencing primer mixture is loaded into 1 reservoir of the primer cartridge and dispensed to 4 different wells. Each primer only anneals to its specific target sequence and will be elongated during the Pyrosequencing reaction. Primers in each MPD mix should be designed and checked to avoid formation of primer-dimers or binding to another PCR template.

Advanced technology, software and chemistry for long and reliable sequence runs

PyroMark Q48 Autoprep features improved chemistry and instrument operation algorithms that significantly increase assay read length and accuracy in base calling, mutation analysis and methylation quantification compared to PyroMark Q96 and PyroMark Q24 systems. Assay read length in previous systems was limited by background peaks

and reduced light signals in the sequencing reaction. The updated PyroMark "Advanced" chemistry and algorithms reduce this background, thereby increasing read length and reliability. Depending on the sequence to be analyzed, highly accurate read lengths of 140 or more bases can be obtained in just a single reaction.

Compatibility of assays among different PyroMark platforms

Previously designed Pyrosequencing assays are readily compatible with the new PyroMark Q48 Autoprep instrument and the “Advanced” chemistry. Data indicate that the same mutation frequencies and methylation quantification results are obtained when the same assay is run on the various PyroMark platforms (Figure 4). The cross-platform

compatibility is also independent of the distance of the analyzed site away from the sequencing primer (data not shown). This is particularly important when analyzing multiple sequence variations in a single run, which is typical for complex mutation assays or methylation analysis of consecutive CpG sites.

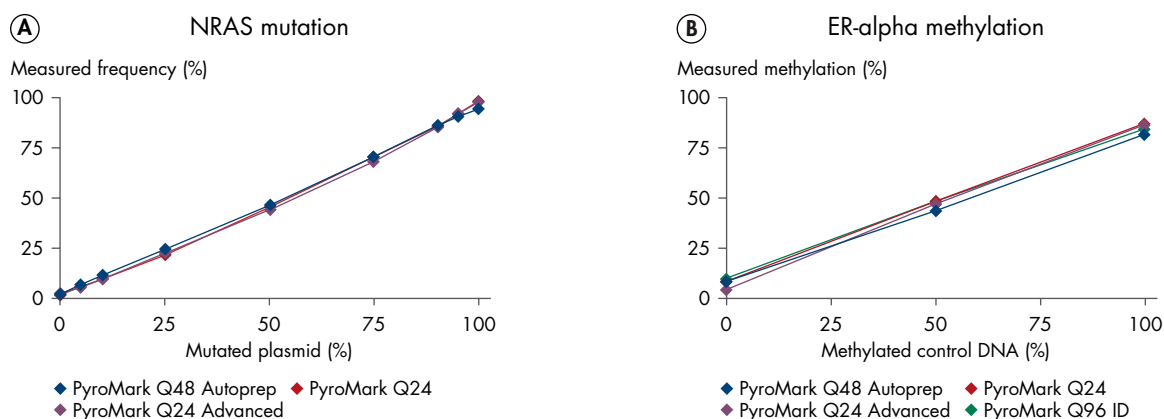


Figure 4. Compatibility among PyroMark platforms. **A** The NRAS Pyro assay was used to measure mutation frequencies in defined mixtures of wild-type and mutated NRAS sequences. The measured mutation frequencies were plotted against the known frequencies. **B** ER-alpha methylation was measured in defined mixtures of methylated and unmethylated control DNA. The measured methylation of one CpG site was plotted against the known methylation percentage.

New disc design enables magnetic template preparation without cross-contamination

PyroMark Q48 Discs are specially designed to automate template preparation and Pyrosequencing in the same instrument without manual user interaction. All buffers used for template preparation are efficiently removed from the

sample and the disc during the run without the risk of any cross contamination from well-to-well of one run or from disc-to-disc between subsequent runs (Figure 5).

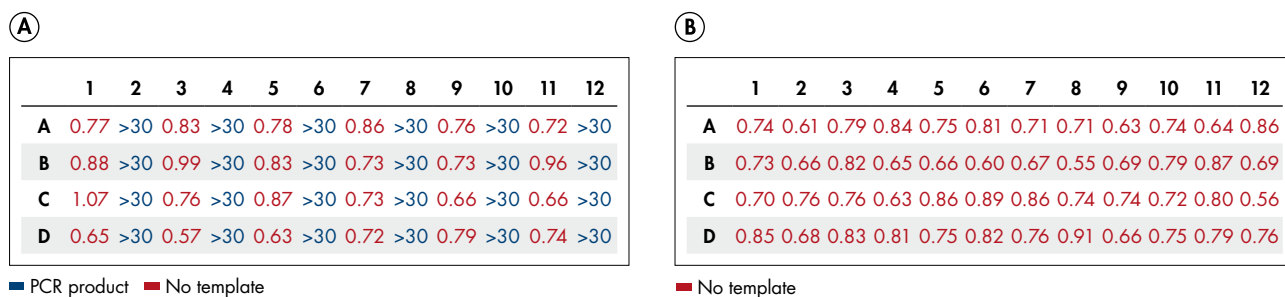


Figure 5. Excluding well-to-well and disc-to-disc cross-contamination. In run **A**, PCR products and non-template controls (NTC) were loaded into a Q48 disc in alternating order. The Pyrosequencing results show no cross-contamination between disc wells. Run **B** was performed with NTC samples only and without replacing the absorber strip from the first run. The results exclude any cross-contamination from one run to another. Results shown in relative light units.

Ordering Information

Product	Contents	Cat. no.
PyroMark Q48 Autoprep	Instrument, software and pipet for advanced Pyrosequencing of 48 samples in parallel	9002470
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
PyroMark Q48 Cartridge Set	Set of 3 cartridges for PyroMark Q48 Autoprep	9024321
PyroMark Q48 Nucleotide Cartridge	Nucleotide replacement cartridge for PyroMark Q48 Autoprep	9024322
PyroMark Q48 Reagent Cartridge	Reagent replacement cartridge for PyroMark Q48 Autoprep	9024323
PyroMark Q48 Primer Cartridge	Primer replacement cartridge for PyroMark Q48 Autoprep	9024324
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 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
PyroMark Q48 Magnetic Beads (300)	Magnetic streptavidin-coated Sepharose® beads for running 300 PyroMark Q48 Autoprep reactions	974203
PyroMark Q48 Software License (1)	1 additional license for PyroMark Q48 Software. Only valid together with PyroMark Q48 Autoprep Software	9024325
PyroMark Q48 Software License (5)	5 additional licenses for PyroMark Q48 Software. Only valid together with PyroMark Q48 Autoprep Software	9024326