PyroMark® PCR Kit

PyroMark PCR Kits (cat. nos. 978703 and 978705) retain full activity at room temperature (15–25°C) for 3 days, but should be stored at –20°C for longer periods.

For more information, please refer to the *PyroMark PCR Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- One primer must be biotinylated at its 5' end for Pyrosequencing® template preparation.
- We recommend PyroMark Assay Design Software 2.0 for primer design.
- If using Q-Solution®, perform parallel amplifications with and without Q-solution.
- We recommend CoralLoad® Concentrate for best PCR performance.

1. Thaw all required solutions (listed in Table 1) and mix each thoroughly. Set up the reaction according to Table 1.
   PyroMark PCR Master Mix contains $MgCl_2$ for a final concentration of 1.5 mM, which provides satisfactory results in most cases. If a higher Mg^{2+} concentration is required, use the included 25 mM $MgCl_2$.
2. Thoroughly mix the reaction mix by gently pipetting up and down and dispense appropriate volumes into PCR tubes.
3. Add template DNA (≤500 ng/reaction) to each PCR tube. We recommend 10 ng human genomic DNA or 10–20 ng bisulfite converted DNA.
4. Program the thermal cycler according to Table 2. If using a thermal cycler without a heated lid, overlay reactions with 100 µl mineral oil.
5. Place the PCR tubes in the cycler and start the cycling program.

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Table 1. Reaction mix composition without template DNA

Component	Volume per reaction	Final concentration
PyroMark PCR Master Mix, 2x	12.5 µl	1x
CoralLoad Concentrate, 10x	2.5 µl	1x
25 mM $MgCl_2$ (optional)	Variable	≥1.5 mM
Q-Solution, 5x (optional)	5 µl	1x
Primer A/Primer B	Variable/Variable	0.2 µM/0.2 µM
RNase-free water	Variable	–
Total volume (after adding template DNA)	**25 µl**	

Table 2. Optimized cycling protocol for PyroMark PCR Master Mix

			Additional comments
Initial PCR activation step	**15 min**	**95°C**	HotStartTaq DNA Polymerase is activated
3 step cycling: Denaturation	**30 s**	**94°C**	
Annealing	**30 s**	**60°C** **56°C**	For genomic DNA For bisulfite converted DNA
Extension	**30 s**	**72°C**	
Number of cycles	**45**		
Final extension	**10 min**	**72°C**	

6. Use 5–10 µl PCR product for Pyrosequencing on the PyroMark Q24 and the PyroMark Q96 MD, and 10–20 µl for the PyroMark Q96 ID.

   Note: We recommend checking your PCR product prior to Pyrosequencing analysis, e.g., by fast analysis on the QIAxcel or by agarose gel analysis.

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