



April 2026

# EGFR Pyro<sup>®</sup> Kit Handbook

For quantitative measurement of mutations in codons 719, 768, 790, 858, and 861, as well as deletions and complex mutations in exon 19 of the human *EGFR* gene using PyroMark<sup>®</sup> Q24, PyroMark Q24 Advanced, and PyroMark Q48 Autoprep

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# Kit Contents

<b>EGFR Pyro Kit</b>	<b>(24)</b>
<b>Catalog no.</b>	<b>10651423</b>
<b>Number of reactions</b>	<b>24</b>
Seq Primer EGFR 719	2 × 24 µL
Seq Primer EGFR Ex19 Del	2 × 24 µL
Seq Primer EGFR 768	2 × 24 µL
Seq Primer EGFR 790	2 × 24 µL
Seq Primer EGFR 858–861	2 × 24 µL
PCR Primer EGFR 719	24 µL
PCR Primer EGFR Ex19 Del	24 µL
PCR Primer EGFR 768+790	24 µL
PCR Primer EGFR 858–861	24 µL
PyroMark PCR Master Mix, 2×	2 × 850 µL
CoralLoad® Concentrate, 10×	1.2 mL
H <sub>2</sub> O	5 × 1.9 mL
Unmethylated Control DNA, 10 ng/µL	100 µL

## Controls

Unmethylated Control DNA is included in the kit as a positive control for PCR and sequencing reactions. This control DNA has a wild-type genotype in the regions sequenced using this kit and is required for adequate result interpretation. Include a sample containing Unmethylated Control DNA for each assay in every Pyrosequencing® run. In addition, include a negative control (no template DNA) in every PCR setup for at least one assay.

# Shipping and Storage

The EGFR Pyro Kit is shipped on dry ice. PyroMark PCR Master Mix, CoralLoad Concentrate, Unmethylated Control DNA, and all primers should be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  upon arrival.

The EGFR Pyro Kit is stable until the kit expiration date when stored under these conditions.

# Intended Use

The EGFR Pyro Kit 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.

# 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](http://www.qiagen.com/safety), where you can find, view, and print the SDS for each QIAGEN kit and components.

## CAUTION



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,<sup>†</sup> or COSHH<sup>‡</sup> documents. Venting for fumes and disposal of waste must be in accordance with all national, state, and local health and safety regulations and laws.

\* OSHA: Occupational Safety and Health Administration (United States of America).

<sup>†</sup> ACGIH: American Conference of Government Industrial Hygienists (United States of America).

<sup>‡</sup> COSHH: Control of Substances Hazardous to Health (United Kingdom).

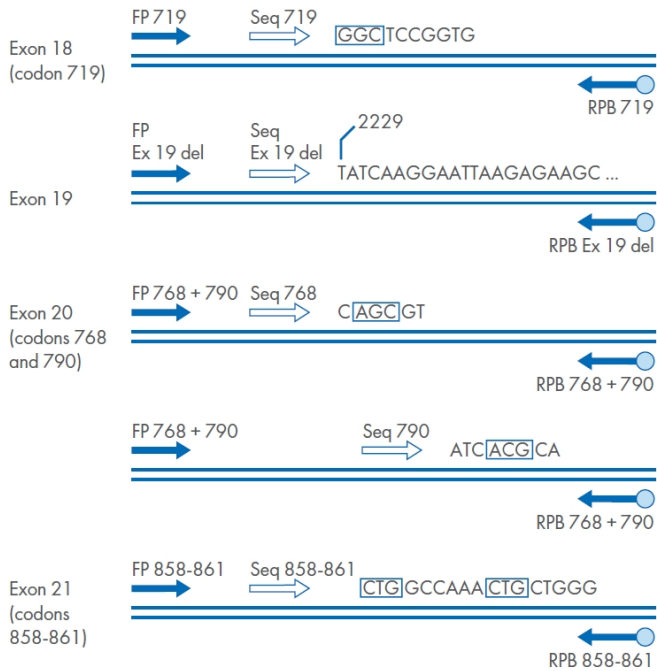
# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the EGFR Pyro Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

## Principle and procedure

The EGFR Pyro Kit is used for quantitative measurement of mutations in codons 719, 768, 790, 858, and 861, as well as deletions and complex mutations in exon 19 of the human *EGFR* gene. The kit consists of four PCR assays: one for detecting mutations in codon 719 (exon 18), one for detecting mutations in codons 768 and 790 (exon 20), one for detecting mutations in codons 858–681 (exon 21), as well as one for detecting deletions and complex mutations in exon 19. The four regions are amplified separately by PCR and sequenced through the defined regions. The amplicon covering codons 768 and 790 is divided into two sequencing reactions. Sequences surrounding the defined positions serve as normalization and reference peaks for quantification and quality assessment of the analysis.



**Figure 1. Illustration of the EGFR assay.** The indicated sequence is the analyzed sequence for a wild-type sample. **FP**, forward PCR primers; **RPB**, reverse PCR primers (B indicates biotinylation); **Seq**, sequencing primers.

All assays are sequenced in the forward direction.

The product consists of a PCR primer mix and a sequencing primer for each assay. The primers are supplied in solution. Each vial contains 24 µL of each primer or primer mix.

# 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.

- DNA preparation reagents
- For further information on required equipment and reagents, refer to the user manual of the instrument being used:
  - PyroMark Q24: See Section 5.3, "Sample Preparation," in the *PyroMark Q24 User Manual* ([www.qiagen.com/HB-0240](http://www.qiagen.com/HB-0240)).
  - PyroMark Q24 Advanced: See Section 5.3, "Sample and Reagent Preparation," in the *PyroMark Q24 Advanced User Manual* ([www.qiagen.com/HB-1341](http://www.qiagen.com/HB-1341)).
  - PyroMark Q48 Autoprep: See the "Preparing Templates and Reagents" section in the *PyroMark Q48 Autoprep User Manual* ([www.qiagen.com/HB-1971](http://www.qiagen.com/HB-1971)).

# Important Notes

- Create an Assay Setup as described below. This must only be done once, before running the *EGFR* Pyro assay for the first time (see “Protocol: Assay and Run Setup”, page 16).
- Ensure that the reactions are thoroughly mixed and prepared and incubated at the recommended temperatures.
- The Sequencing Primer stock solution has a concentration of 10  $\mu\text{M}$ .
  - **PyroMark Q24:** Mix 0.8  $\mu\text{L}$  of undiluted Sequencing Primer stock solution (10  $\mu\text{M}$ ) with 24.2  $\mu\text{L}$  Annealing Buffer to prepare 25  $\mu\text{L}$  of a Sequencing Primer solution at a final concentration of 0.32  $\mu\text{M}$ .
  - **PyroMark Q24 Advanced:** Mix 0.75  $\mu\text{L}$  of undiluted Sequencing Primer stock solution (10  $\mu\text{M}$ ) with 19.25  $\mu\text{L}$  Advanced Annealing Buffer to prepare 20  $\mu\text{L}$  of a Sequencing Primer solution at a final concentration of 0.375  $\mu\text{M}$ .
  - **PyroMark Q48 Autoprep:** Dilute the Sequencing Primer stock solution (10  $\mu\text{M}$ ) to a final concentration of 4  $\mu\text{M}$  by mixing 0.8  $\mu\text{L}$  Sequencing Primer stock solution with 1.2  $\mu\text{L}$  Advanced Annealing Buffer for one reaction.
    - For manual sequencing primer loading, pipet 2  $\mu\text{L}$  of diluted Sequencing Primer (4  $\mu\text{M}$ ) into each well.

**Important:** Wait until the software prompts you to add the Sequencing Primer (4  $\mu\text{M}$ ). Do not pipet the Sequencing Primer (4  $\mu\text{M}$ ) into the wells in the beginning of the run. For more information, see *PyroMark Q48 Autoprep User Manual* ([www.qiagen.com/HB-1971](http://www.qiagen.com/HB-1971)).
    - For automatic sequence primer loading, prepare higher volumes of diluted Sequencing Primer (4  $\mu\text{M}$ ), as required.

## Description of protocols

The first step is to amplify the target DNA by PCR, as described in “Protocol: PCR Using the PyroMark PCR Kit”, page 13. The *EGFR* Pyro Assay and Run should be set up while the PCR is running, following the instructions in “Protocol: Assay and Run Setup,” page 16. After amplification, follow the protocols according to the respective instrument user manual to prepare the sequencing templates for Pyrosequencing analysis:

- *PyroMark Q24 User Manual* ([www.qiagen.com/HB-0240](http://www.qiagen.com/HB-0240)): See Section 5.3, “Sample preparation,” continuing from Section 5.3.3 “Immobilizing the PCR products to beads,” and Section 5.4 “Preparation of PyroMark Gold Q24 Reagents.”
- *PyroMark Q24 Advanced User Manual* ([www.qiagen.com/HB-1341](http://www.qiagen.com/HB-1341)): See Section 5.3, “Sample and reagent preparation,” continuing from Section 5.3.3, “Immobilizing the PCR products to beads.”
- *PyroMark Q48 Autoprep User Manual* ([www.qiagen.com/HB-1971](http://www.qiagen.com/HB-1971)): See “Preparing templates and reagents,” continuing from Section 6.1.5, “Absorber strip”.

Finally, perform the Pyrosequencing run and analyze the data according to the relevant instrument user manual:

- *PyroMark Q24 User Manual* ([www.qiagen.com/HB-0240](http://www.qiagen.com/HB-0240)): See Section 5.5, “Processing a run on the PyroMark Q24 Instrument.”
- *PyroMark Q24 Advanced User Manual* ([www.qiagen.com/HB-1341](http://www.qiagen.com/HB-1341)): See Section 5.4, “Processing a run on the PyroMark Q24 Advanced.”
- *PyroMark Q48 Autoprep User Manual* ([www.qiagen.com/HB-1971](http://www.qiagen.com/HB-1971)): See “Starting a run” and Section 7, “PyroMark Q48 Autoprep Software”.

# Protocol: PCR Using the PyroMark PCR Kit

This protocol describes the setup and cycling conditions for the amplification of DNA using the PyroMark PCR Master Mix (included in kit). The PCR products are subsequently used for detection of mutations in of the *EGFR* gene by Pyrosequencing analysis.

## Important points before starting

- See the *PyroMark PCR Kit Handbook* ([www.qiagen.com/HB-3794](http://www.qiagen.com/HB-3794)) for more detailed information.
- HotStarTaq® DNA Polymerase requires an activation step of 15 min at 95°C (step 5 of the protocol).
- Set up all reaction mixtures in an area separate from areas used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Before opening the tubes containing PCR primers, spin briefly to collect contents at the bottom of the tubes.
- Adjust the concentration of the control and sample DNA, if necessary, to 0.4–2 ng/μL.

## Procedure

1. Thaw the PyroMark PCR Master Mix, CoralLoad Concentrate, and primer solutions.

**Important:** Mix the solutions before use to avoid localized concentrations of salt.

2. Prepare a reaction mix for each PCR primer set according to Table 1.

**Table 1. Preparation of reaction mix for each PCR primer mix**

Component of reaction mix	Volume (µL) per reaction
PyroMark PCR Master Mix, 2	12.5
CoralLoad Concentrate, 10x	2.5
PCR Primer <i>EGFR</i> 719 or PCR Primer <i>EGFR</i> Ex 19 Del or PCR Primer <i>EGFR</i> 768 and 790 or PCR Primer <i>EGFR</i> 856–861	1
RNase-free water	4
<b>Total volume</b>	<b>20</b>

3. Gently pipet the reaction mixture up and down to mix thoroughly, and dispense 20 µL into each PCR tube. It is not necessary to keep PCR tubes on ice, as HotStarTaq DNA Polymerase is inactive at room temperature.
4. Add 5 µL template DNA (2–10 ng of genomic DNA) to the individual PCR tubes, and mix thoroughly.

**Note:** Include a negative control sample (without template DNA) in every PCR setup for at least one assay. In addition, include a sample containing Unmethylated Control DNA for each assay in every Pyrosequencing run (see “Controls,” page 3).

- Program the thermal cycler according to Table 2.

**Table 2. Optimized cycling protocol for PyroMark PCR Master Mix**

	Time	Temperature (°C)	Notes
<b>Initial PCR activation step</b>	15 min	95	HotStarTaq DNA Polymerase is activated by this heating step
<b>3-step cycling</b>			
Denaturation	20 s	95	
Annealing	30 s	53	
Extension	20 s	72	
<b>Number of cycles</b>			<b>42</b>
<b>Final extension</b>	5 min	72	
<b>Hold</b>	∞	4	

- Place the PCR tubes in the thermal cycler and start the cycling program.

**Note:** After amplification, samples can be stored overnight at 2–8°C. For longer storage store at –30°C to –15°C.

- Use 10 µL of PCR product for subsequent Pyrosequencing analysis. We recommend checking the PCR product before Pyrosequencing analysis, for example, by rapid analysis on the QIAxcel® system or by agarose gel analysis. See the *PyroMark PCR Kit Handbook* ([www.qiagen.com/HB-3794](http://www.qiagen.com/HB-3794)) for details. The amplicon lengths are 96 bp for codon 719, 127 bp for codons 768 and 790, 88 bp for codons 858–861, and 126 bp for exon 19.
- Proceed to “Protocol: Assay and Run Setup,” next page.

# Protocol: Assay and Run Setup

These protocols are for setting up the assay parameters and creating a Run Setup for mutation analysis in *EGFR*.

Use default settings in the software for all assay setups if not otherwise stated.

## Procedure

1. Set up the *EGFR* assay by selecting **New AQ** Assay in the PyroMark Q24, PyroMark Q24 Advanced, or PyroMark Q48 Autoprep Software.
2. Table 3 shows the “Sequence to Analyze” for all *EGFR* assays. Type the assay-specific sequence in the “Sequence to Analyze” field of the software.

**Note:** The “Sequence to Analyze” can also be modified after the run (if not locked) to analyze for mutations at different positions in codons 719, 768, 790, 858, and 861, as well as deletions and complex mutations in exon 19. To reanalyze and target additional mutations, go to **Analysis Setup** and change the default “Sequence to Analyze” to one of the additional “Sequence to Analyze” listed in Table 4. Click **Apply**, and then click **To All** when the Apply Analysis Setup window appears.

**Note:** We strongly recommend reanalyzing all samples with no mutation detected using the standard “Sequence to Analyze”, as well as samples that received a “Check” or “Failed” quality assessment or show peaks that do not match the height of the histogram bars. “Check” and “Failed” quality assessments may indicate a mutation that is not addressed by the standard “Sequence to Analyze”, resulting in peak height deviations.

3. Manually enter the assay-specific “Dispensation Order” from Table 3.

**Note:** Do not use the **Generate Dispensation Order** button. Both “Sequence to Analyze” and “Dispensation Order” must be entered in manually.

**Note:** In the histogram of codon 719, move the mouse cursor to the upper end of the bar at dispensation C5 and click while holding down the **Ctrl** button. A small window appears showing the default height of the histogram bar (1.00). Increase the level to 1.04 for “Sequence to Analyze” DGCTCCGGTGC and to 2.04 for “Sequence to Analyze” GSCTCCGGTGC.

In the histogram of codon 790, move the mouse cursor to the upper end of the bar at dispensation T8 and click twice while holding down the **Ctrl** button. A small window appears showing the default height of the histogram bar (1.00). Increase the level to 1.03.

4. Click the toolbar, and save the assay as as *EGFR 719*, *EGFR codon 768*, *EGFR codon 790*, *EGFR codon 858–861*, or *EGFR Exon 19 del*.

**Table 3. Assay setup: “Sequence to analyze” and “Dispensation order” for the EGFR Pyro assays**

Assay	Sequence to analyze	Dispensation order
EGFR 719	DGCTCCGGTGC	ATGTCACCTCGTG
EGFR 768	CAKCGTG	TCGAGTCGAT
EGFR codon 858–861	CKGGCCAAACDGCTGGGT	ATCGTGCAAGCATGCTG
EGFR Exon 19 del	TATCAA[GGAATTAAGAGAAGC] AACATCTCCGAAAGCCAACAAGGA	CTATCACTGTCAAGTCGATCGTCA TCGTCACGC

**Table 4. Common mutations in the human EGFR gene detected by the EGFR Pyro Kit with respective “Sequence to analyze”**

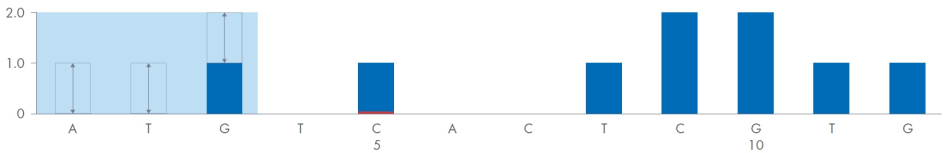
Nucleic acid substitution	Amino acid substitution	Cosmic ID	Sequence to analyze
<b>Exon 18 Codon 719 (GGC)</b>			
AGC*	G719S	6252	DGCTCCGGTGC
TGC*	G719C	6253	DGCTCCGGTGC
GCC	G719A	6239	GSCTCCGGTGC
<b>Exon 20 Codon 768 (AGC)</b>			
ATC*	S768I	6241	CAKCGTG
<b>Exon 20 Codon 790 (ACG)</b>			
ATG*	T790M	6240	ATCAYGCAG
<b>Exon 21 Codon 858 (CTG)</b>			
CGG*	L858R	6224	CKGGCCAAACDGCTGGGT
<b>Exon 21 Codon 861 (CTG)</b>			
CAG	L861Q	6213	CKGGCCAAACDGCTGGGT
CGG	L861R	12374	CKGGCCAAACDGCTGGGT
<b>Del Exon 19†</b>			
2235del15*	E746_A750del	6223	TATCAA [GGAATTAAGAGAAGC] AACATCTCCGAAAGCCAACAAGGA
2236del15*	E746_A750del	6225	TATCAAG [GAATTAAGAGAAGCA] ACATCTCCGAAAGCCAACAAGGA
2239del9	L747_E749del	6218	TATCAAGGAA [TTAAGAGAA] GCAACATCTCCGAAAGCCAACAAGGA
2240del15*	L747_T751del	12369	TATCAAGGAA [TTAAGAGAAGCAACA] TCTCCGAAAGCCAACAAGGA

**Table 4. Common mutations in the human EGFR gene detected by the EGFR Pyro Kit with respective “Sequence to analyze” (continued)**

Nucleic acid substitution	Amino acid substitution	Cosmic ID	Sequence to analyze
2240del18*	L747_P753>S	12370	TATCAAGGAAT [TAAGAGAAGCAACATCTC] CGAAAGCCAACAAGGA

\* For these mutations, the ability to detect different mutation frequencies on the PyroMark Q48 Autoprep was validated.

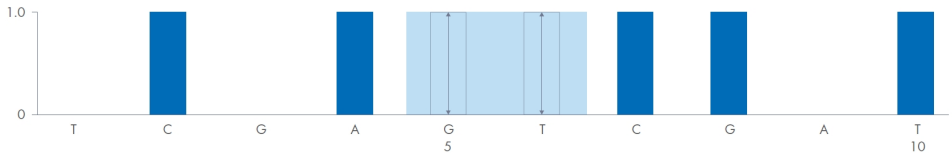
† Sequence to analyze can be easily adapted to different deletions in Exon 19. Use the wild-type sequence TATCAAGGAATTAAGAGAAGCAACATCTCCGAAAGCCAACAAGGAAATCCTCGAT and add squared brackets where the deletion starts and ends.



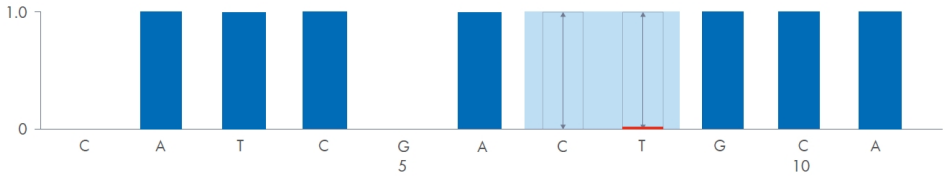
**Figure 2. Histogram for codon 719 (nucleotide 2155) with the “Sequence to Analyze” DGCTCCGGTGC.** The red rectangle at the bottom of the bar at dispensation C5 illustrates the adjustment of heights of histogram bar.



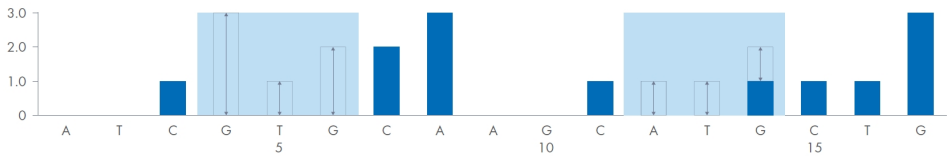
**Figure 3. Histogram for codon 719 (nucleotide 2156) with the “Sequence to Analyze” GSCTCCGGTGC.** The red rectangle at the bottom of the bar at dispensation C5 illustrates the adjustment of heights of histogram bar.



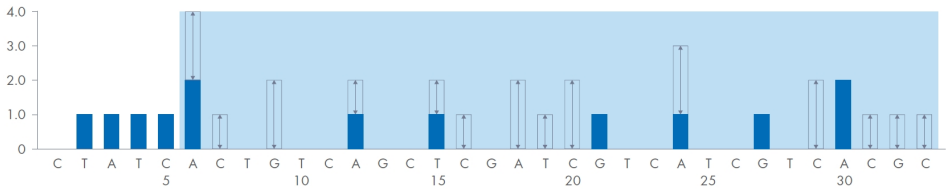
**Figure 4. Histogram for codon 768 (nucleotide 2303) with the “Sequence to Analyze” CAKCGTG.**



**Figure 5. Histogram for codon 790 (nucleotide 2369) with the “Sequence to Analyze” ATCAYGCAG.** The red rectangle at the bottom of the bar at dispensation T8 illustrates the adjustment of heights of histogram bar.



**Figure 6. Histogram for codons 858–861 with the “Sequence to Analyze” CKGGCCAAACDGCTGGGT.**



**Figure 7. Histogram for exon 19 del.**

## Run setup

1. Create a new run file by select **New Run**.
2. Setup the plate by adding the *EGFR* assays to selected wells.
3. Proceed with the Run Setup, preparation of samples and run according to the instructions in *PyroMark Q24 User Manual*, *PyroMark Q24 Advanced User Manual*, or *PyroMark Q48 Autoprep User Manual*.

# 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](http://www.qiagen.com/FAQ/FAQList.aspx) (for contact information, visit [www.qiagen.com](http://www.qiagen.com)).

Refer to the user manual for the PyroMark instrument which is used.

- When using PyroMark Q24, see Section 7, “Troubleshooting Guide,” in the *PyroMark Q24 User Manual*.
- When using PyroMark Q24 Advanced, see Section 7, “Troubleshooting Guide,” in the *PyroMark Q24 Advanced User Manual*.
- When using PyroMark Q48 Autoprep, see Section 9, “Troubleshooting Guide,” in the *PyroMark Q48 Autoprep User Manual*.
- For an assay-specific Troubleshooting Guide, see “Troubleshooting Guide,” in the *EGFR Pyro Handbook* for PyroMark Q24 ([www.qiagen.com](http://www.qiagen.com)).

## Assay-specific troubleshooting

### “Check” or “failed” result

Warning message “Uncertain/Failed due to high peak height deviation at dispensation: 8” appears in the codon 790 assay.

### Comments and suggestions

Background noise at dispensation T8 is below the expected level. Adjust the height of the histogram bar to the default value (1.00).

# Contact Information

For technical assistance and more information, please see our Technical Support Center at [support.qiagen.com](https://support.qiagen.com) or contact one of the QIAGEN Technical Service Departments or local distributors (visit [support.qiagen.com](https://support.qiagen.com)).

# Ordering Information

Product	Contents	Cat. no.
<b>Assays &amp; Controls</b>		
EGFR Pyro Kit	For 24 reactions: Forward primer, Reverse primer, and Sequencing primer for the mutation analysis of <i>EGFR</i> using the PyroMark Q24, PyroMark Q24 Advanced, or PyroMark Q48 Autoprep	970480 10651423
PyroMark Control Oligo	For installation check of the system	979203
<b>PyroMark Q24</b>		
PyroMark Q24	Instrument, for laboratory use only	9001514
PyroMark Q24 Software	Analysis software, for laboratory use only	9019062
<b>PyroMark Q24 Advanced</b>		
PyroMark Q24 Advanced CpG Reagents (4 × 24)	For 4 × 24 samples for use on the PyroMark Q24 Advanced: Enzyme Mixture, Substrate Mixture, Buffers, and Nucleotides for CpG and long-read sequencing runs	970922
PyroMark Q24 Advanced	Instrument, software, and installation for advanced Pyrosequencing analysis of 24 samples in parallel	9002270
<b>PyroMark Q48 Autoprep</b>		
PyroMark Q48 Autoprep	Instrument, software, and pipette	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 Autoprep Starter Kit	PyroMark Q48 Magnetic Beads (300), PyroMark Q48 Advanced CpG Reagents (4 × 48), PyroMark Control Oligo, PyroMark Q48 Discs (50) and PyroMark Q48 Absorber Strips (100)	974230
PyroMark Q48 Advanced Reagents (4 × 48)	Reagents for 4 × 48 PyroMark Q48 Autoprep standard reactions	974002
PyroMark Q48 Advanced CpG Reagents (4 × 48)	Reagents for 4 × 48 PyroMark Q48 Autoprep CpG and long-read reactions	974022

Product	Contents	Cat. no.
PyroMark Q48 Magnetic Beads (300)	Magnetic streptavidin-coated Sepharose beads for running 300 PyroMark Q48 Autoprep reactions	974203

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Support or your local distributor.

# Document Revision History

Date	Description
04/2026	Initial release

### Limited License Agreement for EGFR Pyro Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at [www.qiagen.com](http://www.qiagen.com). Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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