February 2017

RAS Extension Pyro® Plug-in Quick-Start Guide

For installation and use with PyroMark® Q24 Instruments and PyroMark Q24 Software version 2.0

Sample to Insight

QIAGEN

About the RAS Extension Pyro Plug-in

The RAS Extension Pyro Plug-in package contains the following:

- *RAS Extension Pyro Plug-in Quick-Start Guide*
- Two installation files
- Reference report for RAS Extension Pyro Plug-in functionality verification

Note: The RAS Extension Pyro Plug-in is intended to be used only in combination with the dedicated NRAS Pyro Kits and RAS Extension Pyro Kits indicated for applications described in the respective NRAS Pyro Kit and RAS Extension Pyro Kit handbooks.

Installation of the RAS Extension Pyro Plug-in

Important: The RAS Extension Pyro Plug-in must be installed on **PyroMark Q24 instruments** with **PyroMark Q24 Software version 2.0**.

1. Close the PyroMark Q24 Software 2.0 if it is open.
2. Open the installation *.zip file and extract the files.
3. Double-click the setup.exe file.
4. Follow the instructions in the dialog boxes that appear.
5. Start the PyroMark Q24 Software 2.0. The RAS Extension Pyro Plug-in Report now appears under “AQ Add On Reports/RAS Extension” in the “Reports” menu in AQ mode.
6. Verify the Plug-in functionality (see “Verification of the RAS Extension Pyro Plug-in Functionality” below).

Verification of RAS Extension Pyro Plug-in Functionality

Important: The verification should be performed each time new software is installed or upgraded on the computer.

The following steps describe how to verify that the software is working correctly and has not been affected by any changes to the computer.

1. Open the RAS Extension Example run under "Shortcuts/ Example Files/PyroMark Runs/RAS Extension" in the shortcut browser.
2. Perform a "RAS Extension" analysis for all wells as described in "Analysis of a PyroMark Q24 Run" below.
3. Compare the results with the reference report. If the results are identical, correct function of the Plug-in is confirmed.

Analysis of a PyroMark Q24 Run

The following steps describe the mutation analysis of a finished RAS Extension run using the RAS Extension Pyro Plug-in.

1. Insert the USB stick containing the processed run file into the computer's USB port.
2. Move the run file from the USB stick to the desired location on the computer using Windows® Explorer.
3. Open the run file in the AQ mode of PyroMark Q24 Software either by selecting "Open" in the "File" menu or by double-clicking the file ( ) in the shortcut browser.
4. Select "AQ Add On Reports/RAS Extension" from "Reports" in the menu (Figure 1).

Note: Mutations in KRAS codon 61 must be analyzed separately using the KRAS Pyro Plug-in by selecting "AQ Add On Reports/KRAS" from "Reports" in the menu (Figure 1).

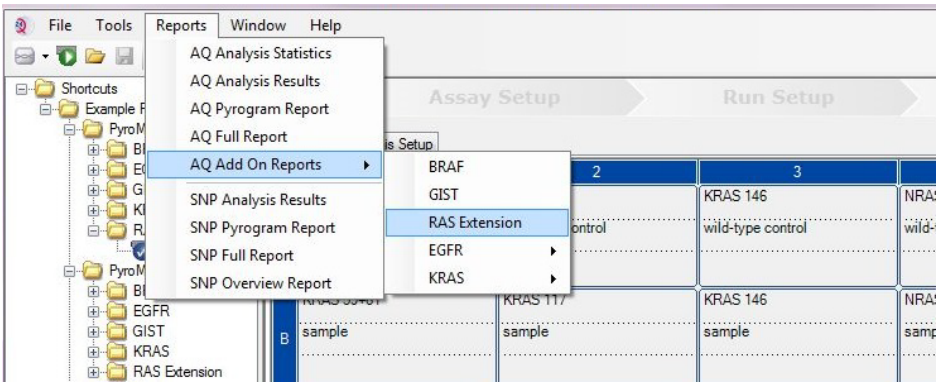

Figure 1. Mutation analysis of a finished RAS Extension run using the RAS Extension Pyro Plug-in.

5. The wells will automatically be analyzed for all mutations listed in Table 1(except KRAS codon 61). The results for all RAS Extension assays will be presented in an overview table (Figure 2), followed by detailed results comprising Pyrograms® and analysis quality.

   Note: Mutations in KRAS codon 61 must be analyzed separately with the KRAS Pyro Plug-in.

Important: The RAS Extension Pyro Plug-in will report the mutation (Table 1) whose expected signal matches the observed Pyrogram best.

Table 1. Mutations analyzed by the RAS Extension Pyro Plug-in

Nucleic acid substitution	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V69)
KRAS codon 59 (GCA)				
175G>A	A59T	0.5	3.5	546
176C>G	A59G	0.5	3.5	28518
KRAS codon 117 (AAA)				
351A>C	K117N	1.0	4.0	19940
351A>T	K117N	3.6	7.1	28519
KRAS codon 146 (GCA)				
436G>A	A146T	2.7	6.6	19404
436G>C	A146P	1.8	4.8	19905
437C>T	A146V	2.1	5.1	19900
NRAS codon 12 (GGT)				
34G>A	G12S	1.4	3.4	563
34G>T	G12C	0.6	2.5	562
34G>C	G12R	0.4	2.4	561
35G>A	G12D	1.8	3.8	564
35G>T	G12V	3.8	8.8	566
35G>C	G12A	0.5	2.5	565
NRAS codon 13 (GGT)				
37G>A	G13S	1.2	3.2	571
37G>T	G13C	1.2	3.2 (4)†	570
37G>C	G13R	0.3	2.3	569
38G>A	G13D	0.8	2.8	573
38G>T	G13V	0.0	2 (5)†	574
38G>C	G13A	0.8	2.8	575
NRAS codon 59 (GCT)				
175G>A	A59T	3.8	6.9	578
176C>G	A59G	0.0	3.0	–

Nucleic acid substitution	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V69)
NRAS codon 61 (CAA)				
181C>A	Q61K	4.1	6.7	580
182A>G	Q61R	0.8	2.2	584
182A>T	Q61L	0.7	2.1	583
183A>T	Q61H	0.4	1.8	585
183A>C	Q61H	5.4	8.0	586
183A>G	Q61Q	2.1	5.8	587
NRAS codon 117 (AAG)				
351G>C	K117N	1.4	4.4	–
351G>T	K117N	3.0	6.0	–
NRAS codon 146 (GCC)				
436G>A	A146T	1.4	4.4	27174
436G>C	A146P	3.5	7.2	–
437C>T	A146V	4.8	7.8	–

* From the Catalogue of Somatic Mutations in Cancer, available online at the Sanger Institute at **www.sanger.ac.uk/genetics/CGP/cosmic**.

† Lowest mutation level in a sample resulting in a measured frequency ≥ LOD.

Summary

Well	Assay Name	Sample ID	Result	Frequency [% units]	Nucleotide Substitution	Amino Acid Substitution	Info
A1	KRAS Codon 59	wild-type control	No mutation detected				
A2	KRAS Codon 117	wild-type control	No mutation detected				
A3	KRAS Codon 146	wild-type control	No mutation detected				
A4	NRAS Codon 12 and 13	wild-type control	No mutation detected				
A5	NRAS Codon 59	wild-type control	No mutation detected				
A6	NRAS Codon 61	wild-type control	No mutation detected				
A7	NRAS Codon 117	wild-type control	No mutation detected				
A8	NRAS Codon 146	wild-type control	No mutation detected				
B1	KRAS Codon 59	sample	Mutation	35,0	175G>A	A59T	
B2	KRAS Codon 117	sample	No mutation detected				
B3	KRAS Codon 146	sample	Mutation	29,6	437C>T	A146V	
B4	NRAS Codon 12 and 13	sample	No mutation detected				
B5	NRAS Codon 59	sample	Mutation	20,5	176C>G	A59G	
B6	NRAS Codon 61	sample	No mutation detected				
B7	NRAS Codon 117	sample	Potential low level mutation	5,0	351G>C	K117N	⚠
B8	NRAS Codon 146	sample	No mutation detected				
C1	KRAS Codon 59	NTC	Failed Analysis				⚠
C2	KRAS Codon 117	NTC	Failed Analysis				⚠

Figure 2. Example results summary from a RAS Extension Pyro Plug-in analysis.

Interpretation of Results and Detection of Low-Level Mutations

It is strongly recommended that a wild-type sample is included in every run for comparison and as a control for background levels.

Important: A "Check" or "Failed" quality assessment can be caused by an unexpected pattern of peaks. This may indicate an unexpected mutation which is not analyzed by the Plug-in Report. These samples should be analyzed manually using the PyroMark Q24 Software with the consideration that they may contain unexpected mutations. See the appropriate NRAS Pyro Kit or RAS Extension Pyro Kit handbook for details.

Important: The Pyrogram should always be compared to the histogram, which is shown in the detailed results of the Plug-in Report and can be displayed in the PyroMark Q24 software by right-clicking in the Pyrogram window. The Pyrogram should be examined for the appearance of unexpected peaks. In case the measured peaks do not match the height of the histogram bars and cannot be explained by rare or unexpected mutations, the result is not a basis for judgment of mutational status. It is recommended to rerun the sample.

Important: Samples with a reported potential low-level mutation (frequency in the range from LOD to LOD + 3 % units) should be rerun in duplicate together with a sample with unmethylated control DNA. A warning will be issued in this case. The sample should only be considered positive for the mutation if both duplicates confirm the result of the original analysis and are visibly different from the normal control. Otherwise, the sample should be judged as wild type.

Important: For closer examination of samples with a reported potential low-level mutation, we recommend to additionally analyze the sample manually in the PyroMark Q24 Software, e.g., for comparison to the mutational frequency in the control sample (see "Protocol 6: Analysis of a PyroMark Q24 run" in the appropriate RAS Extension Pyro Kit handbook for detailed instructions). A measured frequency above LOB in the control sample indicates a higher than usual level of background in the corresponding run, which may impact allele quantification especially for low mutational levels. In this case, reported potential low-level mutations are not a basis for judgment of mutational status and it is recommended to rerun samples with a potential low-level mutation.

Ordering **www.qiagen.com/contact** | Technical Support **support.qiagen.com** | Website **www.qiagen.com**