

February 2016

KRAS Pyro[®] Plug-in Quick-Start Guide

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

About the KRAS Pyro Plug-in

The KRAS Pyro Plug-in package contains the following:

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

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

Installation of the KRAS Pyro Plug-in

Important: The KRAS 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 KRAS Pyro Plug-in Report now appears under "AQ Add On Reports/KRAS" in the "Reports" menu in AQ mode.
6. Verify the plug-in functionality (see "Verification of the KRAS Pyro Plug-in Functionality" below).

Verification of KRAS 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 KRAS Example run under "Shortcuts/ Example Files/PyroMark Runs/KRAS" in the shortcut browser.
2. Perform a "KRAS codon 12 and 13" 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 KRAS run using the KRAS 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/KRAS" and "Codon 12 and 13" or "Codon 61" from "Reports" in the menu (Figure 1).

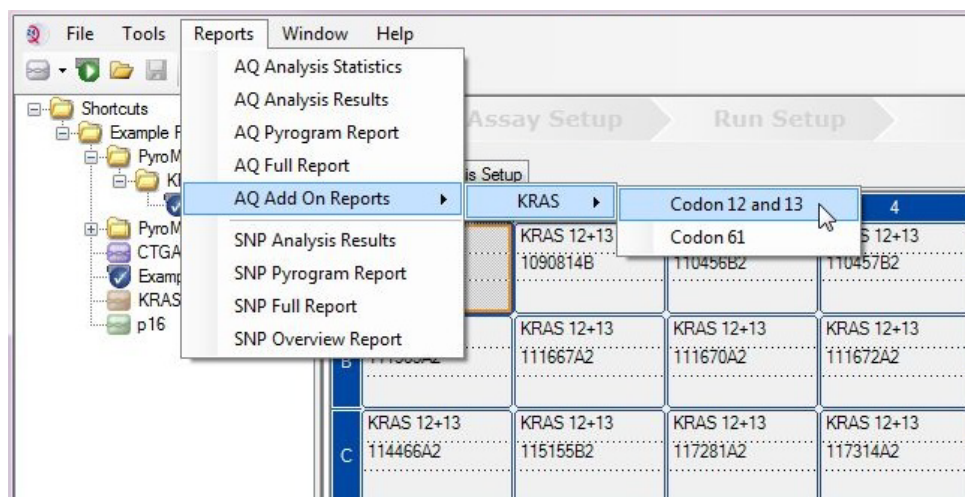


Figure 1. Mutation analysis of a finished KRAS codon 12 and 13 run using the KRAS Pyro Plug-in.

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

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

Table 1. Mutations analyzed by the KRAS Pyro Plug-in

Nucleic acid substitution	Amino acid substitution	LOB (% units)	LOD (% units)	COSMIC ID* (V72)
Codon 12 (GGT)				
GAT	G12D	0.6	2.2	521
GTT	G12V	4.9	8	520
TGT	G12C	0.5	2.1	516
AGT	G12S	0.4	1.9	517
GCT	G12A	0.7	2.3	522
CGT	G12R	0.3	1.8	518
Codon 13 (GGC)				
GAC	G13D	0.3	1.9	532
Codon 61 (CAA), as assayed in reverse orientation (TTG)				
GTG	Q61H	0.8	2.8	554
TAG	Q61L	1.2	3.1	553
TCG	Q61R	1.6	3.5	552
ATG	Q61H	0.7	2.6	555
TTC	Q61E	1.2	3.1	550

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

Summary

NOTE: Only the mutation with the highest frequency is reported.

Well	Sample ID	Result	Frequency [% units]	Nucleotide Substitution	Amino Acid Substitution	Info
A1	106506B1	Mutation	28.8	GGT>AGT	G12S	
A2	1090814B	Wildtype				
A3	110456B2	Potential low level mutation	2.3	GGT>AGT	G12S	⚠
A4	110457B2	Wildtype				
A5	110462A2	Wildtype				
A6	110486A2	Mutation	24.9	GGT>GCT	G12A	
A7	111207A2	Mutation	31.6	GGT>GTT	G12V	
A8	111555A2	Mutation	39.7	GGT>GAT	G12D	
B1	111565A2	Mutation	37.5	GGT>GAT	G12D	
B2	111667A2	Mutation	26.7	GGT>GTT	G12V	
B3	111670A2	Wildtype				
B4	111672A2	Mutation	21.1	GGT>GTT	G12V	
B5	112307A2	Wildtype				
B6	113070A2	Wildtype				
B7	113188A1	Mutation	55.1	GGT>TGT	G12C	
B8	113200A1	Wildtype				
C1	114466A2	Wildtype				

Figure 2. Example results summary from a KRAS 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 KRAS 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.

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