QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
Germany

January 16, 2018

Urgent Field Safety Notice: *therascreen* KRAS Pyro Kit (24) CE (REF 971460), *therascreen* BRAF Pyro Kit (24) CE (REF 971470), *therascreen* EGFR Pyro Kit (24) CE (REF 971480), *therascreen* GIST RapidScreen Pyro Kit (REF 971510), *therascreen* NRAS Pyro Kit (24) CE (REF 971530), *therascreen* RAS Extension Pyro Kit (REF 971590), PyroMark Gold Q24 Reagents (5 x 24) CE (REF 971802)

Dear *therascreen* Pyrosequencing customer,

This urgent field safety notice is to inform you that we have identified an increased number of cases with altered ratios in the Pyrogram peak patterns for *therascreen* Pyrosequencing assays. Use of the product LOTs listed below is associated with an elevated invalidity and re-testing rate.

In addition, use of the product LOTs listed below also creates an elevated risk of A59T false positive results for the Pyrosequencing analysis of NRAS codon 59 with the *therascreen* RAS Extension Pyro Kit (REF 971590).

For other mutations in the *therascreen* RAS Extension Pyro Kit or other *therascreen* Pyro kits there is no evidence of increased risk for such false positive results.

According to our investigation the issue is related to certain LOTs of PyroMark Q24 Gold reagents (Mat. No. 1055272) as listed below. These are contained in several LOTs of the *therascreen* Pyro Buffers and Reagents box (Mat. No. 1063948) delivered with the CE-marked IVD *therascreen* Pyro kits as listed in the header above. All LOTs of products were released according to QC specifications.

According to our records, you have received at least one kit of the affected LOTs of products.

Overview of affected material:

Material	LOT	Contained in	LOTs
PyroMark Gold Q24 Reagents (Mat. No. 1055272)	157010641	*therascreen* Pyro Buffers and Reagents (Mat. No. 1063948)	157013230, 157013013
	157025615		157027507
	157027017		157028499, 157028495, 157030047
	157030977		157035803, 157033230

PROM-11774-001

Description of issue

In Pyrosequencing runs performed with the affected material, the ratio of the peak height for dATP peaks in Pyrograms is artificially increased while in parallel the peak height for dGTP may be decreased. This may result in an altered ratio of peak heights and consequently leads to an incorrect determination of allele mutation frequencies. If the determined allele mutation frequency exceeds the limit of detection (LOD), this may cause an erroneous manual or automated calling of the corresponding “potential low level mutations” or in some cases of a “mutation” by the Pyrosequencing software. The error can additionally influence the quality scoring via the corresponding algorithms of the Pyrosequencing software. The error affects independently wild type (WT) controls and unknown samples. For all assays except NRAS59 this does not, to our knowledge, lead to a potentially increased false positive rate when evaluating Pyrosequencing results according to the corresponding Instructions for Use. However, for all assays an increased need for retesting can occur in these cases.

For “mutation” results of the NRAS codon 59 mutations (A59T) in affected LOTs: The probability of calling a mutation A59T with mutation allele frequencies below 15% is increased while the WT control is valid (no mutation detected) and the quality scoring is not impaired.

Actions to be taken by the customer/user

- Please ensure that the corresponding assay Plug-in reports for automated result interpretation are used. These Plug-ins also provide example Pyrograms to ease identification of deviations in the peak pattern. Up-to-date Plug-ins can be retrieved directly on our corporate QIAGEN website (**https://www.qiagen.com/**) on the related product page(s), by going to the “Product Resources” tab, under the “Analysis Software” section.

- **If you have any remaining stocks of the affected LOTs**, do not use them any longer. Please contact our technical service for a free of charge replacement of remaining Pyromark Gold Reagents LOTs. Please provide the LOT No. of the Pyromark Gold as well as REF No. and kit name of the *therascreen* kit. Please also provide an example pyro run file with unknown samples and WT controls labeled accordingly.

- **If you have used the affected LOTs,** we strongly recommend a review of the corresponding results. In order to identify the altered peak ratio issue in your Pyrosequencing runs, please, use the following criteria:

    - A higher number than usual of WT control DNA samples above the LOD resulting in potential low-level mutation or mutation is an indication for the error.

- A higher number than usual of samples flagged with impaired quality assessment such as “check“ or “failed” according to the automatic scoring algorithm of the software is an indication for the error.
- A higher than average detection frequency of “potential low-level mutation” or “mutation” calls for unknown samples compared to the natural prevalence of this mutation among the cancer disease and ethnicity investigated. The natural prevalence of some mutations analyzed by Pyrosequencing assays can be below 5%. Any increased frequency of mutation calls for such rare variants (e.g. several such calls over a short period of time) may be indicative of the issue.
- The calling of several (2 or more) “mutations” or “potential low-level mutations” in different codons of the same gene from the same sample may be indicative for the issue.

In case you identify the issue by one or several of the above criteria we strongly recommend re-evaluation of the affected Pyrosequencing results. Please follow strictly the interpretation of analysis results as described in the corresponding Instructions for Use of the *therascreen* Pyro kits and pay attention to the below observations and corresponding actions:

Re-evaluation	**Observation**	**Action for samples with an A59T mutation call below 15% mutation frequency in NRAS59**	**Action for any other *therascreen* Assays**
1. Re-evaluation of WT controls independent of the mutation result for an unknown sample	Detected mutation frequency for any WT control DNA in a run is above the LOD. This results in a potential low-level mutation or mutation call for this WT control.	According to the Instructions for Use the run is invalid for this assay. For any re-testing please use exchanged PyroMark Q24 Gold Reagents LOTs.	According to the Instructions for Use the run is invalid for this assay. For any re-testing please use exchanged PyroMark Q24 Gold Reagents LOTs.
2. Re-evaluation of unknown samples with mutation allele frequencies between LOD and 15% mutation frequency but with valid WT control results	1) Measured peaks in the Pyrogram do not match the height of the histogram bars and cannot be explained by rare or unexpected mutations. If you are using the automated report Plug-ins, you can compare each individual Pyrogram with an example Pyrogram to identify such deviations. 2) An increased background for a mutation as detected by allele frequencies for the WT control DNA between the Limit of Blank (LOB) and LOD as listed in the handbook for this mutation.	Consider an increased possibility of false positive results. Confirm all positive A59T “mutation” and “potential low-level mutation” results below 15% allele frequency and for which at least one of the observations apply by re-testing with exchanged Pyromark Gold Reagents LOTs. If re-testing is not possible, assess each case individually.	If analyzed according to Instructions for Use no additional re-testing for confirmation is necessary.

- Review this notice with your laboratory/medical director
- IMPORTANT: Forward this information to all individuals and departments within your organization using the above listed kits. If you are not the end user, please forward this notice to the product end user. Commercial partners must forward this notice to their customers
- Dispose of the affected product in accordance with your national and local safety and environmental regulations
- Complete Acknowledgement of Receipt attached to this letter
- Commercial partners:
    - Cease distribution of the products listed in this notice
    - Forward this notice to your customers
    - Follow-up on the Acknowledgements of Receipt with your customers

Completion of the Acknowledgement of Receipt

To ensure that all affected users are notified, and according to applicable national statutory provisions, we are obliged to provide the authorities with proof of market notification. Therefore, please complete and sign the included Acknowledgement of Receipt form by January 29th, 2018 and email it to: **quality.communications@qiagen.com**.

Actions that have been initiated at QIAGEN

The remaining stock of the affected material has been blocked in all QIAGEN warehouses. As immediate corrective action, an additional quality control test has been included for all PyroMark Gold Reagent LOTs. The root cause of the issue is currently under investigation.

We sincerely apologize for any inconvenience this may cause and thank you in advance for your understanding and collaboration.

If you have any questions, please contact your local QIAGEN Technical Services Department.

Please visit the following webpages for contact information:

QIAGEN Subsidiaries : **https://www.qiagen.com/about-us/contact/global-contacts/subsidiaries/**

QIAGEN Commercial Partners and Importers:

https://www.qiagen.com/about-us/contact/global-contacts/distributors-and-importers/

With kind regards,

QIAGEN

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Acknowledgement of Receipt

(Please complete form using block letters and indicate with an "X" that you followed each instruction)

We have taken the necessary actions as suggested by this notice:

- ☐ We did not use the remaining stocks of the affected LOTs.
- ☐ If we have used the affected LOTs, we did a review of the corresponding results.
- ☐ We considered a retesting of A59T mutation samples with mutation frequencies below 15%.
- ☐ We reviewed this Notice with our laboratory/medical director.
- ☐ The information was forwarded to all individuals and departments within our organization using these products.
- ☐ We disposed of the affected product in accordance with national and local safety and environmental regulations.
- ☐ Commercial Partners: we ceased the distribution of the affected products. This information was forwarded to our customers/partners. We followed-up on the Acknowledgements of Receipt with our customers.

Laboratory name:	
Address:	
Contact name:	Title:
Email address:	Phone number:
Signature:	Date: