February 2023

Acknowledgment of Receipt Form

Please complete this form and email it to **quality.communications@qiagen.com** by March 1, 2023 using the following acknowledgment text (it will be equivalent to your signature):

I hereby acknowledge that I have received, read, and understood the included Important Note for FGFR RGQ RT-PCR Kit (24) (ref. no. 8747010) dated February 2023. We will take the necessary actions as suggested by this notice.

Laboratory name:

Address:

Contact name:

Title:

Phone number:

Date:

Signature:

February 2023

Important Note

FGFR RGQ RT-PCR Kit (24), ref. no. 8747010; Confirmatory retest of positive results might be required

Dear valued FGFR RGQ RT-PCR Kit customer,

QIAGEN has identified an increased occurrence rate of false positive results obtained with the FGFR RGQ RT-PCR Kit (lot nos.172017806 and 169047135). For a proportion of samples with the individual target result "Amplification Detected", the result could be a false positive result.

Please note that the cDNA samples can be stored at –30 to –15°C for up to 10 days. Disregard the statement, "samples can be stored at –30 to –15°C for up to 30 days", mentioned in the *FGFR RGQ RT-PCR Kit Instructions for Use (Handbook)*, December 2019, (page 30, point number 21).

Potential risks associated with the issue

The issue can potentially lead to a false positive sample result.

Detailed description of the issue

Only samples with an individual target result: "Amplification Detected" (as per Analysis Report), are affected. In the affected runs, the Run Controls pass correctly (leading to a valid result for the sample), while an artefact in one or more of the mutation assays might cause an incorrect valid mutation positive result for individual samples. The software for the interpretation of all mutations claimed in the assay does not distinguish such artefacts from a real amplification obtained with a valid mutation positive sample. An affected sample can be identified by the manual analysis of the amplification curve shape as described on the next page of this notice "Actions to be taken by the customer".

The investigation at QIAGEN demonstrated that all targets/Amino acid variants/Fusion IDs are affected, except for the Amino acid variant p.S249C (c.746C>G on Exon 7).

As an interim solution, the newly manufactured batches with lot numbers from 175014234 and higher, will undergo additional testing (until the issue is resolved or the investigation is completed) to ensure a minimum specificity of 95%. If a sample result obtained is positive, a retest may be required as described in this Important Note in order to mitigate the risk of a false positive result. Upon retest, the minimum specificity increases to 99%.

At this stage, any positive result showing the individual target result: "Amplification Detected" for targets other than p.S249C (c.746C>G on Exon 7) should not be used. The amplification curve shape should be analyzed in order to identify false positive results, as described in the section "Actions to be taken by the customer" below.

The following are acceptable results:

- Sample Results with "No Amplification Detected" are not affected by this issue and are the correct result.
- "Amplification Detected" results with individual target result for p.S249C (c.746C>G on Exon 7) are not affected and can be regarded as correct. This includes results for samples without or with additional individual other target results.

Actions to be taken by the customer

- For the continued use of FGFR RGQ RT-PCR Kit (ref. no. 8747010) consider the following criteria:
    - Samples with "No Amplification Detected" should be regarded as correct and no further actions are needed.
    - Samples with the individual target result "Amplification Detected" for p.S249C (c.746C>G on Exon 7) can be regarded as correct. This also includes samples with additional alterations detected.
    - For all other "Amplification Detected" results except for p.S249C (c.746C>G on Exon 7), a manual analysis of the amplification curve should be performed.

Table 1. Overview of affected results

Individual target result	Affected by the issue	Action to be taken by the customer
"-" No amplification detected for p.R248C (c.742C>T), p.G370C (c.1108G>T), p.S249C (c.746C>G), p.Y373C (c.1118A>G), FGFR3:TACC3v3, FGFR3:BAIAP2L1, FGFR2:CASP7, FGFR3:TACC3v1 and FGFR2:BICC1	No	n/a (correct result)
"Amplification Detected" for p.S249C (c.746C>G)	No	n/a (correct result)
"Amplification Detected" for p.S249C (c.746C>G) and additional individual target results	No	n/a (correct result)
One or more individual target results that don't include p.S249C (c.746C>G)	Yes	Manually analyze the amplification curve shape in order to distinguish artefacts from real amplification and thus to identify false positive results. For further information, refer to "Instructions for manual curve analysis" and the attachment "Identification of Sloping Baselines in the FGFR RGQ RT-PCR System"

Instructions for manual curve analysis

The Rotor-gene Assay Manager is unable to differentiate between the two curve shapes leading to the generation of C_T values for both false-positive and genuine positive cases. However, the false positive results can be identified due to the presence of an anomalous curve shape (for example see Figure 2) that is not representative of a true-amplification curve (for example see Figure 1). For all details, see the attachment "Identification of Sloping Baselines in the FGFR RGQ RT-PCR System"

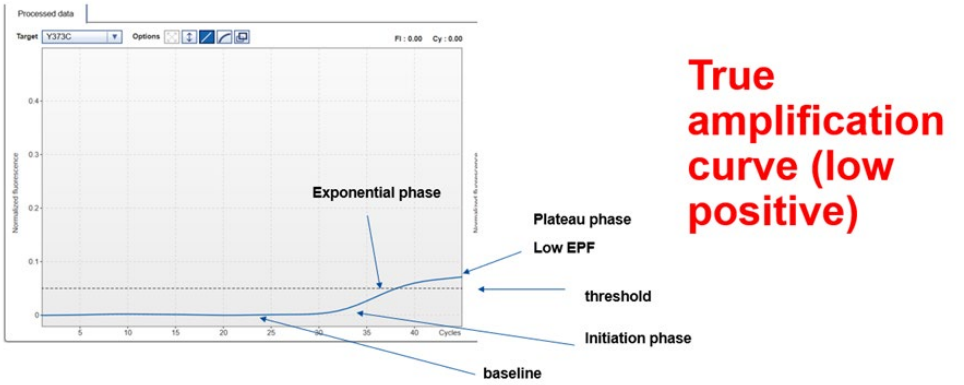

Figure 1. True amplification curve with exponential phase, low positive result.

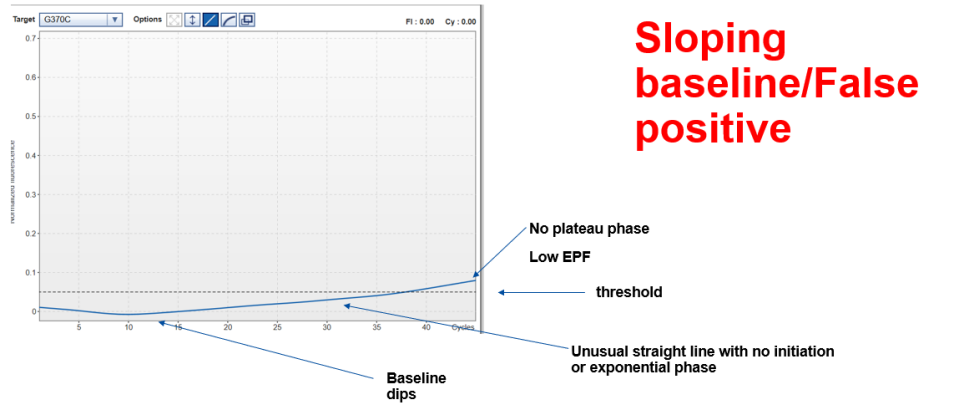

Figure 2. False positive result due to an artefact, sloping baseline without exponential phase.

Please use the following instructions for your review:

- All Individual target result “Amplification Detected” samples listed on the RGAM run report (except p.S249C (c.746C>G)) must be checked;
- Assess if the alteration is a “True Positive” or “False-Positive” by checking the curve shape;
- In samples where multiple alterations are identified, the curve shape of each alteration must be checked;
- For detailed instructions, refer to the attachment "Identification of Sloping Baselines in the FGFR RGQ RT-PCR System";
- Forward this information to all individuals and departments within your organization who are using REF 8747010 FGFR RGQ RT-PCR Kit;
- If you are not the end user, please forward this notice to the product end user;
- Review this notice with your laboratory director;
- Complete the “Acknowledgement of the Receipt Form” attached to this letter by March 1, 2023, and email it to **quality.communications@qiagen.com**

Actions taken by the QIAGEN

QIAGEN is working on identifying and correcting the root cause of false positivity. Any update on mitigation measures will be promptly communicated. In the interim, we are providing kits with this Important Note.

If you have any questions or concerns, please contact your local QIAGEN Technical Service Department through any of the following:

QIAGEN Subsidiaries
www.qiagen.com/de/about-us-old/contact/global-contacts/subsidiaries

QIAGEN Commercial Partners and Importers
www.qiagen.com/de/about-us-old/contact/global-contacts/distributors-and-importers

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

With kind regards,

QIAGEN