June 2021

Important Note

therascreen® PIK3CA RGQ PCR Kit, REF 873121

Dear valued *therascreen* PIK3CA RGQ PCR Kit customer,

As part of our ongoing market surveillance process, QIAGEN has identified that the *therascreen* PIK3CA RGQ PCR Kit may generate false Q546R mutation positive results caused by non-specific molecular interactions within the Q546R reaction.

In these cases, the run controls for the Q546R reaction pass validity checks correctly. The software for the interpretation of PCR run data and identification of the Q546R mutation is currently not able to differentiate between the signal created by the non-specific interaction and that created by genuine amplification from a valid mutation positive sample. Consequently, Q546R false positive results are reported to the system operator.

As a result of the preliminary investigation performed at QIAGEN, the likelihood for any Q546R false mutation positive result was evaluated as higher than previously observed and described within the Instructions For Use.

The performance of other mutation detection assays within the kit is not affected.

Potential risks associated by this issue

The issue can potentially lead to a false positive *PIK3CA* Q546R mutation detected result, which could subsequently be reported by the laboratory. Receipt of such a false positive result by the clinician who ordered the test could lead to incorrect treatment decisions being made that could significantly impact patient health, including inappropriate treatment of a patient with PIQRAY® (alpelisib).

Actions to be taken by the customer/user

- For patient samples where a *PIK3CA* Mutation Detected Result is obtained, disregard Q546R positive results.
- Cease to report patient samples where a Q546R mutation result is obtained as *PIK3CA* Mutation Detected. Information about individual target results is specifically obtained from the "Individual target result" column of the Rotor-Gene Assay Manager v2.1 result table.

Rotor-Gene Assay Manager v2.1 result table for a *therascreen* PIK3CA RGQ PCR Kit *PIK3CA* Mutation Detected result

Pos.	Sample ID	Type	Sample comment	Overall sample result	Flags	Output	Ct	Value	Individual target result
17, 18, 19, 20, 21, 22	Sample 1 REP 1	Test		PIK3CA Mutation Detected	–	T1_Control	29.54	–	Valid
						T4_Q546R ΔCt		4.89	Q546R Mutation Detected

- If multiple mutations including Q546R are detected, disregard **only** the Q546R result. Continue to consider all other results as valid and report them accordingly.
- Continue to use all supplied reagents and perform testing as described in the kit handbook, but disregard Q546R positive results generated for patient samples as described in the previous bullet points. The *therascreen* PIK3CA RGQ PCR Kit software requires valid run control data from the Q546R reaction for the overall test to be valid. Therefore, for the purpose of run validity, Q546R control data must still be produced.

- Forward this information to all individuals and departments within your organization who are using the *therascreen* PIK3CA RGQ PCR Kit REF 873121. If you are not the end-user, please forward this information to the product end-user.
- Review this Important Note with your laboratory/medical director.

Retesting of samples or re-analysis of existing PCR data

QIAGEN is currently unable to provide instructions to customers which describe any procedures that can be used to re-analyze data and determine whether Q546R mutation detected results are attributable to detection of genuine Q546R mutations, or are False Positive results. Therefore, QIAGEN do not recommend that customers perform re-testing of samples or re-analysis of existing PCR data at this time using the *therascreen* PIK3CA RGQ PCR kit.

Actions taken by QIAGEN

QIAGEN is now revising the Instructions for Use for the *therascreen* PIK3CA RGQ PCR Kit, as described in this notice, to reduce any risk resulting from non-specific molecular interactions within the Q546R reaction leading to the generation of Q546R false mutation positive results.

QIAGEN is also updating the *therascreen* PIK3CA FFPE and Plasma Assay Profile software packages to resolve this issue. You will be informed as soon as the updated software is available, and provided with instructions for updating the *therascreen* PIK3CA assay profiles. Until then, we advise you to disregard and cease to report mutation detected results generated by the *PIK3CA* Q546R reaction, as outlined above.

Translation of this Important Note

QIAGEN provides the printed Important Note in the English language. Translation of the Important Note are electronically available in a portable document format (PDF). You may access the translated documents in the Product Resources tab of the corresponding product page in **www.qiagen.com**.

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/support**, call 080-000-7145, or contact one of the QIAGEN Technical Service Departments (**TechService-KR@qiagen.com**) or local distributors (visit **www.qiagen.com**).

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

With kind regards,

QIAGEN

www.qiagen.com