therascreen® EGFR RGQ PCR Kit

Performance Characteristics

therascreen EGFR RGQ PCR Kit, Version 1, REF 870111

Cut-offs

171 FFPE samples were tested using a method following guidance in NCCLS EP17-A (2004). Data from 159 samples were used in establishing cut-offs for the kit. The control reaction C_T range was established as 23.00 to 30.69 C_T. The cut-off values were established and are shown in Table 1.

Table 1. Cut-off ΔC_T values for each EGFR mutation reaction

Assay	Cut-off ΔC_T value
T790M	6.38
Deletions	9.06
L858R	8.58
L861Q	9.26
G719X	9.31
S768I	9.26
Insertions	7.91

* Acceptable ΔC_T values are equal to or less than the values shown.

Limit of detection (LOD)

To determine the LOD for the *therascreen* EGFR RGQ PCR Kit, a sample set was developed by mixing synthetic mutant DNA with wild-type genomic DNA to simulate a range of mutation percentages for each of the 29 mutations. The LOD of each assay is defined as the percent mutation at which 95% of replicates were determined positive by the *therascreen* EGFR RGQ PCR Kit. The LOD values are given in Table 2. For the multiplex assays, which detect multiple mutations (G719X, the deletions and the insertions), the value for the reaction that gave the highest LOD is given.

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Table 2. LODs for each of the seven EGFR mutation assays

Mutation	Percentage mutation detectable (%)
T790M	7.02
Deletions	1.64
L858R	1.26
L861Q	0.50
G719X	5.43
S768I	1.37
Insertions	2.03

Precision

To determine precision of the *therascreen* EGFR RGQ PCR Kit, a sample set was developed by mixing synthetic mutant DNA with wild-type genomic DNA to simulate a low level of mutation percentage for each of the seven mutation assays. Precision was assessed by testing samples at one testing site, using multiple kit batches, operators and runs over different days, with two replicates of each sample. The variation seen, in terms of estimated standard deviation from Variance Component Analysis, was less than 1 ΔC_T and can be used as an estimate of precision (Table 3).

Table 3. Results of within-laboratory tests*

Assay	Percentage testing mutation positive	Estimate of standard deviation (ΔC_T)
T790M	100%	0.33
Deletions	100%	0.40
L858R	100%	0.45
L861Q	100%	0.49
G719X	97.9%	0.59
S768I	97.9%	0.31
Insertions	97.9%	0.38

* 93 replicates were tested for each mutation.

Reproducibility

Reproducibility was assessed by testing high mutation level samples in a background of wild-type genomic DNA at three testing sites, using multiple kit batches, operators and runs over different days, with two replicates of each sample. For all seven mutation assays 96.1–100% of mutant DNA samples tested mutation-positive. Wild type samples tested mutation-negative in all assays at all sites.

Effect of input DNA concentration

To determine the effect of changing input DNA concentration on the results produced by the *therascreen* EGFR RGQ PCR Kit close to the LOD, a sample set for all 29 mutations was developed by mixing synthetic mutant DNA with wild-type genomic DNA to produce samples at low, medium, and high total input DNA levels

The high and low levels of input DNA were targeted to represent the control assay C_T value range (23.50 to 29.50).

An assessment of the input DNA data set (29 mutations, at concentrations close to the LOD and at three different input DNA levels) revealed a 95.44% mutation positive rate.

These data indicate that varying the level of input DNA, within the working range of the assay, does not impact the ΔC_T or mutation call of a sample.

Interfering substances

The effect on kit performance of components that could potentially carry over from the QIAGEN® QIAamp® DNA FFPE Tissue Kit during the processing of FFPE samples was assessed.

Formalin, paraffin wax, xylene, ethanol, buffer ATL, proteinase K, buffer AL, wash buffer AW1, and wash buffer AW2 were used at the highest ("worst case") concentrations expected (assuming that each washing or purification step in the extraction kit protocol resulted in a reduction in the concentration component by 1 log).

The study used three times LOD samples rather than a much higher level of mutation to ensure that potential interference could be detected.

A difference in ΔC_T of $\geq$3 standard deviations (taken from the precision study) between the "test" and the "control" (i.e., no interfering substance) was considered to indicate a potential interference.

None of the potential interfering substances evaluated had a ΔC_T change of $\geq$1 standard deviation when compared to controls.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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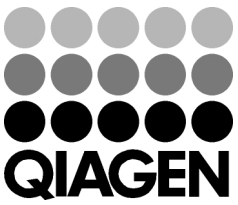

Sample & Assay Technologies