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QIAamp® DSP DNA FFPE Tissue Kit Instructions for Use (Performance Characteristics)

Version 2

IVD

For In Vitro Diagnostic Use
For use with QIAamp DSP DNA FFPE Tissue Kit

CE

REF 60404

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R1 Performance Characteristics available electronically and can be found under the resource tab of the product page on **www.qiagen.com**.

Sample to Insight

General Introduction

The QIAamp DSP DNA FFPE Tissue Kit is a system that uses silica-membrane technology (QIAamp technology) for isolation and purification of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) biological specimens.

It is intended for manual sample preparation purposes and gives no test results, qualitative or quantitative.

Performance Characteristics

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. They have been established for the QIAamp DSP DNA FFPE Tissue Kit in conjunction with exemplary FFPE-embedded tissue types and exemplary downstream applications. However, methods for isolating nucleic acids are used in conjunction with different biological specimen and as a front-end for multiple downstream applications. Performance parameters such as cross contamination or run repeatability and reproducibility need to be established for any such workflow as part of the downstream application development. Therefore, it is the responsibility of the user to validate the whole workflow to establish appropriate performance parameters.

Basic performance and compatibility to different downstream applications

Downstream analysis

Eluted genomic DNA is ready for use in different downstream assays, including a variety of in vitro diagnostic downstream assays. Refer to the relevant QIAGEN® kit handbook for more information on specific system performance.

Yield of purified DNA

Formalin-fixed, paraffin-embedded (FFPE) samples may exhibit a high degree of tissue heterogeneity. In addition, tissue surface area is highly variable in FFPE samples, leading to variable quantity and quality of extracted DNA. Therefore, the user should optimize the number of sections, section thickness, and section surface area for their sample of interest and any procedures used in their laboratory to obtain DNA of suitable quantity and quality for the specific downstream applications.

If the kit is being used in conjunction with a QIAGEN downstream application, refer to the relevant handbook for instructions.

Insufficient tissue dehydration during FFPE tissue preparation, placing too much paraffin with the sample to extraction tube, using lower-purity ethanol (not molecular-biology-grade) than recommended or the retaining of xylene or ethanol in the sample may lead to suboptimal extraction and low DNA quantity and quality.

Repeatability

Repeatability was evaluated using 6 FFPE cell lines generated from human cells fixed in formalin and embedded in paraffin. The samples were tested with QuantiTect® SYBR® Green master mix and β-actin gene-specific primers together with the Rotor-Gene® Q real-time PCR cycler. PCR reactions were performed for a 174 bp fragment and for a 218 bp fragment of the human β-actin gene.

For the statistical analysis, 72 data points for each fragment size were used. Statistical analysis included the calculation of the standard deviation (SD) and upper and lower 95% confidence limits. The variation was estimated using variance component analysis as the standard deviation for the 218 bp fragment (SD: 0.342 CT; lower 95% confidence limit: 0.291; upper 95% confidence limit: 0.413). This can be used as an estimate of repeatability for the extraction process. Variation estimated for 174 bp fragment was 0.258 CT; lower 95% confidence limit: 0.220; upper 95% confidence limit: 0.312.

Reproducibility

Assessment of reproducibility was performed across three laboratories using 3 clinical FFPE specimens containing non-small cell lung cancer (NSCLC) tissue: one harboring a deletion 6223 mutation, one harboring an L858R mutation, and one harboring a wild-type (WT) specimen. The clinical FFPE specimens were selected on the basis of their known mutation status according to Sanger sequencing.

For each of the mutant clinical FFPE specimens, 48 sequential FFPE sections were randomized into pairs to be used in an extraction and divided into three batches, one batch per test site.

Extractions were carried out in duplicate at each test site. Each site used one unique lot of the QIAamp FFPE DNA DSP Kits for extraction. Sample assessment and mutation assessment were carried out using the *therascreen*® EGFR RGQ PCR Kit across all three sites. Samples were tested on 3 non-consecutive days over a period of 6 days. Each specimen was tested 6 times at each site giving a total of 18 data points per specimen.

For all samples, over all three sites, 100% correct mutation calls were demonstrated.

Linearity

The QIAamp DSP DNA FFPE Tissue Kit can be used for isolation of DNA from different types of tissue. A linear range should be established as per customer requirements and validated for the particular use. Different linear ranges are expected for different tissue types, depending on the tissue load into the system, as well as tissue characteristics.

Interfering substances

The QIAamp DSP DNA FFPE Tissue Kit can be used for isolation of DNA from different types of tissue. Potentially interfering substances can originate from different sources, for example, natural metabolites specific for the tissue type and organ, metabolites produced during pathological conditions, substances introduced during patient treatment, or substances ingested by the patient.

Interfering substances testing has been performed using the QIAamp DSP DNA FFPE Tissue kit for sample preparation in conjunction with exemplary downstream applications for an assessment of the quality of the extracted nucleic acids. Examples for tested diagnostic QIAGEN kits are listed in Table 1.

However, different downstream applications may have different requirements with respect to purity (i.e., absence of potential interfering substances) and interferents present in the specific sample may be diverse. Therefore, the identification, testing and control of relevant interfering substances also needs to be established as part of the specific diagnostic workflow involving the QIAamp DSP FFPE Tissue Kit and the specific downstream application.

Table 1. Downstream Assay Interfering substances study

Diagnostic Kit	Interferents Tested	Conclusion
therascreen PIK3CA RGQ PCR Kit	Paraffin Wax Xylene Ethanol Buffer ATL Proteinase K Buffer AL Buffer AW1 Buffer AW2 Hemoglobin	Five mutant samples (each representing one of the assays in the PIK3CA Kit) and one WT sample were spiked with 9 potential interfering substances and tested for their effect on mean ΔCt and mutation call. The data from this study show that the interferents tested had no effect on mutant or WT samples at the concentrations used. Where a significant difference was observed, this was within 3x intermediate precision of the assay and was therefore within the inherent variability of the assay. All mutation calls in both mutant and WT samples were as expected. The data observed in this study show that the study met the acceptance criteria.
therascreen KRAS RGQ PCR Kit	Paraffin Wax Xylene Ethanol Buffer ATL Proteinase K Buffer AL Buffer AW1 Buffer AW2	This study was designed to evaluate the effects of potential interfering substances on the performance of the KRAS kit. For mutant samples, the goal was to demonstrate that the mean assay values in samples with an interfering substance did not differ significantly from those without the interfering substance. For WT samples, the goal was to demonstrate that presence of an interfering substance should not cause false positive results. There were two assay/interfering substance combinations that resulted in false positive results. However, these were both in the low level of the xylene with no comparable false positives in the high level samples. Both of these goals were met, confirming the hypothesis that no substance from the QIAamp DSP DNA FFPE Tissue Kit at the concentrations in normal use interferes with the ability of the KRAS kit to distinguish between mutation positive and mutation negative samples.
therascreen EGFR RGQ PCR Kit (EGFR Kit)	Paraffin Wax Xylene Ethanol Buffer ATL Proteinase K Buffer AW1 Buffer AW2	The objective of this study was to verify the effect of potential interfering substances used in the extraction process on the performance of the *therascreen* EGFR RGQ PCR Kit (EGFR Kit) when used on the QIAGEN Rotor-Gene Q MDx platform (RGQ). Eight FFPE standard samples representing each of the 7 EGFR mutation assays plus one wild-type (WT) were chosen for this study. The estimated differences in mean ΔCt values for each of the mutant FFPE Standards between each of the two levels of interferents, and the "Blank" replicates were either not-significantly different from zero or considered small with a value of less than 1Ct. All mutant replicates had a mutation call of mutation detected at each of the low and high interferent levels for all interferents. All WT replicates had a sample mutation status of mutation not detected at each of the low and high interferent levels for all interferents. The study confirmed that the reagents used in the FFPE Extraction Kit do not affect the performance of the EGFR Kit.
therascreen KRAS RGQ PCR NSCLC Kit	Paraffin Wax Xylene Ethanol Buffer ATL Buffer AL Buffer AW1 Buffer AW2 Buffer ATE	The study was designed to demonstrate that the presence of a potentially interfering substance (from the QIAamp DSP DNA FFPE Tissue Kit (FFPE Extraction Kit)) would not produce any false positive or false negative results for the KRAS System NSCLC kit; that is, the mutation calling would be affected or cause the system to "fail-safe" by producing an invalid sample status. Eight potentially interfering substances from the DNA extraction process were identified. Each substance was tested against 8 FFPE cell lines, representing each of the 7 mutations detected by the KRAS Kit NSCLC Kit, and a WT sample. The mutation samples were tested at a level corresponding to approximately 3 times the limit of detection (3 x LOD). The study demonstrated that the substances tested did not have any adverse effect on the performance of the assay at the 1x level of interferent; the correct mutation call was always called and the presence of the interfering substance did not have a statistically significant effect on difference in ΔCt on the majority of sample conditions tested (58 out of 64 conditions, at 1x level). For the 6 samples that did show a statistically significant difference, the observed difference in the means for each sample was within the study acceptance criterion of ±2 x SD (SD estimate taken from the repeatability and reproducibility study report). The study also demonstrated that the assay was tolerant to higher levels of each of the substances than the expected carry over, i.e., the correct mutation call was given when the interfering substance was present at 10x the highest expected concentration.

Refer to kit handbooks for more information on interfering substances in specific QIAGEN downstream applications.

Cross-contamination

To assess the level of cross-contamination, two FFPE cell line NSCLC samples were used: WT and the FFPE cell line sample harboring the exon 21 L858R mutation. The study aimed to mimic the situation whereby samples containing a high level of mutation can cross-contaminate other samples within the extraction procedure. DNA purification was conducted to challenge the procedure by purifying DNA from L858R mutant samples positioned next to WT samples, using one lot of reagents. The cross-contamination was assessed using the *therascreen* EGFR RGQ PCR Kit. The results showed no cross-contamination within the entire system.

QIAamp DSP DNA FFPE DNA eluate performance in Pyrosequencing® and qPCR-based assays

DNA isolated from FFPE tissue was diluted to a DNA concentration of 2 ng/µl for analysis using the *therascreen* EGFR Pyro Assay. In all runs used for determination of performance characteristics, the signal was over 30 RLU (relative light units) for all codons and all samples had a correct medical outcome for the mutation analysis.

DNA isolated from FFPE tissue of patients with colorectal cancer, non-small cell lung cancer, and breast cancer was used directly in the *therascreen* KRAS RGQ PCR Kit, the *therascreen* EGFR RGQ PCR Kit, the KRAS RGQ PCR NSCLC Kit, and the *therascreen* PIK3CA RGQ PCR Kit. The Ct values of the DNA extracted using the QIAamp DSP DNA FFPE Tissue Kit were within in the working range parameters defined for each assay and detailed in the respective handbooks.

Eluate stability

Eluate stability will depend on the content and type of co-purified impurities (related to tissue type), elution volume, and storage conditions. We recommend that users establish the eluate stability as per their particular requirements.

If the kit is being used in conjunction with a QIAGEN downstream application, refer to the relevant kit handbook for instructions. An exemplary stability verification study has demonstrated that DNA extracted from FFPE tissue samples is suitable for use with the *therascreen* KRAS RGQ PCR Kit when stored for up to 7 days at 4°C with additional storage at –20°C for up to a combined total of five weeks with multiple freeze/thaw cycles.

Symbols

The following symbols appear in this document. For a full list of symbols used in the instructions for use or on the packaging and labeling, please refer to the handbook.

Symbol	Symbol definition
CE	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
IVD	In vitro diagnostic medical device
REF	Catalog number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Manufacturer

Revision History

Revision	Description
R1, June 2022	Version 2, Revision 1 • Update to version 2 for compliance to IVDR • Sections for Interfering substances, Cross contamination, Eluate stability and Compatibility to downstream applications added

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.