Performance Characteristics

QIAamp® DSP DNA FFPE Tissue Kit, Version 1 REF 60404

Version management

This document is the QIAamp DSP DNA FFPE Tissue Kit Performance Characteristics, Version 1, R3.

CE IVD 	Check availability of new electronic labeling revisions at **www.qiagen.com/HB-0414** before test execution. The current revision status is indicated by the issue date (format: month/year).

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 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.

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.

Repeatability

Repeatability was evaluated using six FFPE cell lines generated from human cells fixed in formalin and embedded in paraffin. The samples were tested with QuantiTect® SYBR® Green mastermix 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 C_T; 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 C_T; lower 95% confidence limit: 0.220; upper 95% confidence limit: 0.312.

Reproducibility

Assessment of reproducibility was performed across three laboratories using three clinical FFPE specimens containing non-small cell lung cancer (NSCLC) tissue: one harboring a deletion 6223 mutation, one harboring a L858R mutation and one 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 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 three non-consecutive days over a period of six days. Each specimen was tested six 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, e.g., 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. Due to the complexity of potential interfering substances and different sensitivity of specific downstream applications, we recommend that users assess the effect of the interfering substances for their own systems and validate the method of control of interference in their specific diagnostic downstream application.

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: wild-type 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 wild-type 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®

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.

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.

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.